BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.

REMARKS/ARGUMENTS

Upon entry of this amendment, claims 1 and 17-19 will be amended, and claims 20-22 will be added, whereby claims 1-20 will be pending, with claim 1 being the sole independent claim.

Reconsideration and allowance of the application are respectfully requested.

Claim of Foreign Priority

Applicants express appreciation for the acknowledgment of the claim of foreign priority as well as receipt of the all of the certified copies of the priority documents. In order that the record is clear, Applicants note that this is a national stage application so that the indication should, in fact be that copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau.

Disclosure Statements And Considered Information

Applicants express appreciation for the Examiner's confirmation of consideration of the Information Disclosure Statements filed August 7, 2000 and July 12, 2002 by forwarding initialed copies of the Forms PTO-1449 submitted therewith with the present Official Action.

With regard to the initialed Forms PTO-1449, Applicants note that the Examiner has crossed through the three Japanese documents that are cited on the Form submitted on August 7, 2000, and has indicated that the Japanese documents will not be considered until a translation is provided to the Examiner.

With respect to consideration of the Japanese documents, Applicants note that the Examiner has initialed consideration of the family member U.S. patents, i.e., U.S. Patent No. 5,340,824 which is a family member of JP 5-507918A, and U.S. Patent No. 4,227,915 which is a family member of JP 54-151966A, as well as the English abstract for JP 55-15455A. Still further, the Notification of Acceptance of Application under 35 U.S.C. 371 indicates that copies of the Japanese documents have been received along with the International Search Report. Therefore, the Examiner is required to indicate consideration of these documents as having been cited in the International Search Report, which is in English.

In view of the above, Applicants respectfully request that the Examiner also confirm consideration of the cited Japanese documents, as required by MPEP 609. For the Examiner's convenience, another copy of the Form PTO-1449 is submitted herewith, and the Examiner is requested to forward a completely initialed Form PTO-1449 with the next communication from the Patent and Trademark Office.

Still further, Applicants note that the Examiner has cited WO 97/02666 on the Form PTO-892. Clearly, this citation is not correct as the document is directed to a different technology. It appears that this document should be WO 87/02666 which is cited in the Information Disclosure Statement of August 7, 2000. Accordingly, Applicants are submitting this information herein to ensure that the record is clear.

Response To Restriction Requirement

The Restriction Requirement has been modified based upon the Election with Traverse filed February 2, 2004 and several telephone conversations on April 8, 2004 with Applicants' representative Arnold Turk. In particular, the Examiner was concerned that the Restriction Requirement was not adequately set forth, and inquired whether the requirement could be modified in the Official Action so as to include the elected species in order to advance prosecution of the application. Applicants agreed to the Examiner's request to advance the prosecution of the application, but requested that the restriction requirement be clarified in the next Office Action in order that the requirement can be reviewed and once again traversed. Applicants further note that the present Office Action sets forth a new and very lengthy Restriction Requirement wherein Group I has been modified to include the elected species and preferred compound elected by Applicants.

With respect to the lack of unity of invention requirement, according to guidelines for examining Markush type claims, once a species is found to be allowable over the prior art, the Examiner is supposed to examine the next species in the claims. In the instant Office Action, the Examiner has not examined the next species. Therefore, the Examiner is respectfully requested to examine the next species in the claims in accordance with Patent and Trademark Office practice.

Still further, at least claim 19 should be examined with the elected species because claim 19 is directed to the ligand which includes the elected species.

Moreover, a lack of unity of invention is supported in the requirement by the assertion that the substituents vary greatly and that the claims do not contain a special technical feature which

defines a contribution over the prior art. The Examiner asserts that WO 01/25200, demonstrates that the X-QCR1R2 tail determines the utility of the molecule because when it is changed the utility of the compound is changed. The Examiner has not supplied a copy of WO 01/25200, but has included on the PTO Form-892 a citation to the Chemical Abstracts of the document. In this regard, the lack of unity of invention assertion does not point to any specific disclosure of WO 01/25200 to support the naked allegations set forth in the requirement. The Examiner is reminded that the burden is on the Patent and Trademark Office to establish a lack of unity of invention, and a lack of unity of invention has not been established in the instant situation.

Accordingly, withdrawal of the requirement and a search of each of the indicated inventions is respectfully requested.

Response to Objection To Claim 18

In response to the objection of claim 18 as being a substantial duplicate of claim 17, Applicants note that this claim further defines the medicament composition of claim 17. However, to advance prosecution of the application, Applicants have amended claim 18 to be directed to a method for therapeutic treatment of a disease caused or promoted by nerve controlling function of a sigma ligand. Moreover, claim 20 has been added directed to a method for preventive treatment of a disease caused or promoted by nerve controlling function of a sigma ligand. Accordingly, this ground of objection should be withdrawn.

Response to 35 U.S.C. 112, First Paragraph, Rejection

Claims 1-18 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The rejection asserts that a salt, hydrate thereof and a solvate are not the same chemical species as the compound depicted in claim 1. The Examiner contends that in the absence of how to make hydrates and solvates of compounds of formula 1 and, in the absence of a delineation of what salts, hydrates and solvates of the compounds depicted in claim 1 are being claimed, there is no umbrella coverage springing forth from the few examples of salts, hydrates and solvates depicted at pages 22 and 23 of the specification.

In response to this ground of rejection, Applicants respectfully submit that that one having ordinary skill in the art would readily understand that the invention includes salts, hydrates and solvates of the disclosed compounds. Applicants note that such various forms of compounds are within the skill of the art, and detailed descriptions thereof are not required.

If this ground of rejection is maintained, the Examiner is respectfully requested to specifically indicate how the description of compounds in Applicants' specification fail to comply with the written description requirement, especially when one following the guidance provided in Applicants' disclosure would readily understand salts, hydrates and solvates that are within Applicants' invention.

Response To Rejection Under 35 U.S.C. 112, First Paragraph, Rejection Regarding Enablement

Claims 1-18 are rejected under 35 U.S.C. 112, first paragraph, because the Examiner asserts that the specification, while being enabling for X equal to benzisoxazol-3-yl in the compound depicted in claim 1, is asserted to not reasonably provide enablement for X equal to all heteroaryl groups, Y equal to any heteroaryl groups, or heteroaryl substituted alkyl groups, or R4 and R5 coming together to form any 5 to 7 membered heterocyclic group together with intervening atoms, and does not provide enablement for the use of the compound to prevent or treat any disease caused or promoted by the nerve controlling function of a sigma ligand. Still further, the rejection asserts that it is also not established in the art to utilized pharmaceutical compositions to present disease.

In this ground of rejection, it is asserted that none of the publications discussed in the rejection have the benzothiazoline ring and have not been examined for their affinity for the sigma binding site. Moreover, it is asserted that in the absence of a showing of correlation between all the diseases disclosed as capable of treatment by the compound of claim 1 and the inhibition of Sigma-2 receptor, one of skill in the art is unable to fully predict possible results from the administration of the compound of claim 1 due to the unpredictability of the role of Sigma-2 receptor, i.e., whether promotion or inhibition would be beneficial for the treatment of the diseases.

In response, the Examiner is reminded that the burden is not on Applicants to establish that the claims are enabled, but is on the Examiner to support an enablement rejection using technical

arguments. See, for example, "Training Materials for Examining Patent Applications with Respect to 35 U.S.C. Section 112, First Paragraph -- Enablement Chemical/Biotechnical Applications" and In re Marzocchi, 439 F.2d 220, 224, 169 USPQ 367, 369 (CCPA 1971).

In particular, it is noted in in Marzocchi, in reversing this rejection, the Court noted that the Patent Office should not be concerned with the breadth of the claims per se and that the burden of showing lack of enablement is on the Patent Office:

> Turning specifically to the objections noted by the board as indicated above, it appears that these comments indicated nothing more than a concern over the breadth of the disputed term . . . . The only relevant concern of the Patent Office under these circumstances should be over the truth of any such assertion.
>
> As a matter of Patent Office practice, then, a specification disclosure which contains a teaching of the manner and process of making and using the invention in terms which correspond in scope to those used in describing and defining the subject matter sought to be patented must be taken as in compliance with the enabling requirement of the first paragraph of 112 unless there is reason to doubt the objective truth of the statements contained therein which must be relied on for enabling support. . . .
>
> [I]t is incumbent upon the Patent Office, whenever a rejection on this basis [lack of enablement] is made, to explain why it doubts the truth or accuracy of any statement in a supporting disclosure and to back up assertions of its own with acceptable evidence or reasoning which is inconsistent with the contested statement.

Id. at 369-70 (emphasis in original). Therefore, the burden of showing lack of enablement is on the Patent Office.

In the present case, Applicants use language in the specification which is commensurate in scope with the claims to describe the enablement of the invention. Moreover, the specification, for example, at the top of page 3 indicates that, "These data suggest that the selective sigma 2 ligand is useful for the treatment of irritable bowel syndrome." Moreover, the Examples such as page 80

show a high affinity for the sigma binding site so that the compounds of the present invention are useful as sigma ligands in the therapeutic and/or preventive treatment of various kinds of diseases and symptoms in which sigma ligands are involved.

Still further, Applicants present herewith copies of the following documents, i.e.,, Kinney et al., European Journal of Pharmacology 294 (1995) 547-553 and Perregaard et al., J. Med. Chem. 1995, 38, 1998-2008.[1] Applicants note that Kinney et al. disclose that sigma 2 binding sites may be a novel therapeutic target for irritable bowel syndrome, such as discussed in the final paragraph of the paper. Moreover, Perregaard et al., disclose that the sigma 2 receptors play a role in anxiolytic-like effects, such as at the top of page 2003, the right-hand column.

Therefore, Applicants have provided sufficient guidance in the originally filed application and the art is of such a nature that one having ordinary skill in the art would be capable of practicing Applicants' invention without undue experimentation. Therefore, the claims comply with the requirements of 35 U.S.C. 112, first paragraph..

---

1 The documents are being submitted in accordance with MPEP 609(C)(3) as part of Applicants' reply to the Office Action in support of an argument so that the requirements of 37 C.F.R. 1.97 and 1.98 need not be met, and the information is being submitted as part of the record with the reply for the Examiner's consideration with Applicants' reply.

It is respectfully submitted that the Patent and Trademark Office has failed to overcome Applicants' showing of enablement and has failed to meet its burden of producing evidence or sound scientific reasoning demonstrating lack of enablement. Of course, Applicants have not disclosed working examples of every compound. To do so would require that the specification include a multitude of examples. What Applicants have done, which is in accordance with the requirements of 35 U.S.C. 112, is to present the concept of their invention, which has been defined in the originally filed specification and claims, and to provide representative examples, so as to be in compliance with the best mode requirement of 35 U.S.C. 112, first paragraph. Thus, not only have Applicants provided a broad description of the intended parameters of their invention, but have also described working examples of their invention.

Applicants are entitled to claims that is commensurate with their invention. As stated in In re Angstadt and Griffin, at page 218:

> Appellants have apparently not disclosed *every* catalyst which will work; they have apparently not disclosed *every* catalyst which will not work. The question, then, is whether in an unpredictable art, section 112 requires disclosure of a test with *every* species covered by a claim. To require such a complete disclosure would apparently necessitate a patent application or applications with "thousands" of examples or the disclosure of "thousands" of catalysts along with information as to whether such exhibits catalytic behavior resulting in the production of hydroperoxides. More importantly, such a requirement would force an inventor seeking adequate patent protection to carry out a prohibitive number of actual experiments. This would tend to discourage inventors from filing patent applications in an unpredictable area since the patent claims would have to be limited to those embodiments which are expressly disclosed. A potential infringer could readily avoid "literal" infringement of such claims by merely finding another analogous catalyst complex which could be used in "forming hydroperoxides".

As in In re Angstadt and Griffin, Applicants have provided sufficient guidance for one having ordinary skill in the art to make and use Applicants' invention without undue experimentation. Applicants are not required to provide working examples of each of their compounds, to show treatment of each disease or to provide the theory behind each treatment. In the instant situation, Applicant has provided sufficient guidance for the claimed invention to enable one having ordinary skill to make and use the same, and the enablement rejection should therefore be withdrawn.

In view of the above, Applicants respectfully submit that the claims are enabled, and the enablement rejection should be withdrawn.

Response To Rejection Under 35 U.S.C. 112, Second Paragraph

In response to the rejection of claims 1-8 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite, Applicants respectfully submit the following.

Applicants respectfully submit that the claims definitely recite Applicants' invention prior to the instant amendment. However, in an attempt to advance prosecution of the application, the claims have been amended herein. For example, claims 1, 17 and 19 have been amended to more clearly recite the salt, hydrate and solvent thereof using alternative language. Claims 17 and 18 have been amended to utilize pharmaceutical composition language and to avoid use of substance as suggested by the Examiner.

Regarding composition claim 17, Applicants respectfully submit that the claims is definitely directed to a composition that includes the recited compound, or a salt thereof or a hydrate thereof or a solvate thereof as an active ingredient.

Moreover, regarding claim 1, Applicants respectfully submit that one having ordinary skill in the art would readily understand the meaning of "with other intervening atoms' in that intervening atoms can be present. Therefore, if this ground of rejection is maintained, the Examiner is respectfully requested to more clearly point out what is considered to be the indefiniteness associated with this language.

Accordingly, this ground of rejection should be withdrawn.

CONCLUSION

In view of the foregoing, the Examiner is respectfully requested to reconsider and withdraw the objections and rejections of record, and allow each of the pending claims.

Applicants therefore respectfully request that an early indication of allowance of the application be indicated by the mailing of the Notices of Allowance and Allowability.

Should the Examiner have any questions regarding this application, the Examiner is invited to contact the undersigned at the below-listed telephone number.

Respectfully submitted,
Jean-Philippe ROCHER, et al.

Bruce H. Bernstein
Reg. No. 29,027

July 21, 2004
GREENBLUM & BERNSTEIN, P.L.C.
1950 Roland Clarke Place
Reston, VA 20191
(703) 716-1191

Form PTO-1449 U.S. Department of Commerce Patent and Trademark Office	Atty. Docket No. P19428	Serial No. 09/530,580
INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Use several sheets if necessary)	Applicant Jean-Philippe ROCHER et al.	
	Filing Date November 4, 1998	Group Unknow

U.S. PATENT DOCUMENTS

EXAMINER INITIAL		DOCUMENT NUMBER	DATE	NAME	CLASS	SUBCLASS	FILING DATE IF APPROPRIATE
		4 2 1 5 1 1 9	07/29/80	MENTRUP et al.			
		5 3 4 0 8 2 4	08/23/94	GUEREMY et al.			
		4 2 2 7 9 1 5	10/14/80	D'AMICO			

FOREIGN PATENT DOCUMENTS

		DOCUMENT NUMBER	DATE	COUNTRY	CLASS	SUBCLASS	TRANSLATION YES	TRANSLATION NO
		0 0 9 2 3 9 1	10/26/83	E. P. O.				
	8	7 / 0 2 0 3 5	04/09/87	W. I. P. O.				
	8	7 / 0 2 3 5 9	04/23/87	W. I. P. O.				
	8	7 / 0 2 6 6 6	05/07/87	W. I. P. O.				
	5	- 5 0 7 9 1 8	11/11/93	JAPAN				
	5	4 1 5 1 9 6 6	11/29/79	JAPAN				
	5	5 / 1 5 4 5 5	02/02/80	JAPAN				

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, Etc.)

	1	Ucar et al., "2(3H)-benzoxazolone and 2(3H)-benzothiazolone derivatives: Novel, potent and selective σ_1 receptor
		ligands", Eur. J. Pharmacol., 335, No. 2/3 pp 267-273 (1997).
	2	English Language Abstract of JP 55-15455.

EXAMINER	DATE CONSIDERED

*EXAMINER: Initial if citation considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

P19428.P05

Sheet 1 of 1

OIPE JUL 21 2004 PATENT & TRADEMARK OFFICE

Form PTO-1449 U.S. Department of Commerce Patent and Trademark Office	Atty. Docket No. P19428	Serial No. 09/530,580
INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Use several sheets if necessary)	Applicant Jean-Philippe ROCHER et al.	
	Filing Date October 10, 2000	Group 1614

U.S. PATENT DOCUMENTS

EXAMINER INITIAL		DOCUMENT NUMBER	DATE	NAME	CLASS	SUBCLASS	FILING DATE IF APPROPRIATE

FOREIGN PATENT DOCUMENTS

		DOCUMENT NUMBER	DATE	COUNTRY	CLASS	SUBCLASS	TRANSLATION YES	TRANSLATION NO
	9	6/05185	02/22/96	W.I.P.O.				

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, Etc.)

		1	Huseyin UCAR et al., "2(3H)-benzoxazolone and 2(3H)-benzothiazolone derivatives: Novel, potent and selective σ receptor ligands", European Journal of Pharmacology, 335, pp. 267-273 (1997).

EXAMINER	DATE CONSIDERED

*EXAMINER: Initial if citation considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

ELSEVIER

European Journal of Pharmacology 294 (1995) 547–553

ejp

σ_2 Site-mediated inhibition of electrically evoked guinea pig ileum longitudinal muscle/myenteric plexus contractions

Gene G. Kinney *, Eric W. Harris, Ranjit Ray, Thomas J. Hudzik

Astra Arcus USA, Department of Biology, P.O. Box 20890, Rochester, NY 14602, USA

Received 1 May 1995; revised 13 September 1995; accepted 14 September 1995

Abstract

Functional and binding studies were performed in order to characterize the relative efficacy and affinity of a number of compounds that bind to σ sites. The ability of σ site ligands to inhibit electrically evoked contraction of the guinea pig ileum longitudinal muscle/myenteric plexus preparation was compared to the affinities of these compounds for σ_1 sites (assessed by displacement of [^{3}H](+)-pentazocine) and σ_2 sites (assessed by displacement of [^{3}H]1,3-di-*o*-tolylguanidine (DTG) in the presence of 5 μM dextromethorphan). It was shown that the rank order of potencies for suppression of electrically evoked contractions of guinea pig ileum perfectly matched the rank order of affinities of these compounds for the σ_2 binding site, while correlating poorly with the σ_1 binding site. In addition, no significant correlations were found between the efficacy of the tested compounds to inhibit contraction of the guinea pig ileum preparation and previously reported affinities for muscarinic, dopamine D_2 or MK-801 binding sites. Thus, the present study represents the first functional bioassay selectively sensitive to agents interacting with the σ_2 receptor subtype binding site, and provides a means with which to further elucidate the functional role of σ_2 sites.

Keywords: σ Binding site; Smooth muscle; Ileum; σ Receptor subtype; (Guinea pig); (Rat)

1. Introduction

σ Binding sites were first postulated in 1976 as a subtype of opiate receptor (Martin et al., 1976). σ Sites were later identified in the guinea pig brain using radiolabeled *N*-allylnormetazocine (SKF-10,047) (Su, 1982), and more recently using haloperidol (Contreras et al., 1987), 3-(3-hydroxyphenyl)-*N*-(1-propyl)piperidine ((+)-3-PPP) (Gundlach et al., 1986), *N*,*N*′-di(*o*-tolyl)guanidine (DTG) (Weber et al., 1986), and (+)-pentazocine (De Costa et al., 1989). These studies have found that σ sites are widely distributed throughout the brain and periphery leading to speculation that this system may play a role in psychosis (Su, 1993; Walker et al., 1990), motor dysfunction (Matsumoto et al., 1989, 1990; Walker et al., 1990), emetic processes (Hudzik, 1991), and hepatic (Musacchio et al., 1988; Walker et al., 1990), endocrine (Su et al., 1988; Walker et al., 1990; Wolfe et al., 1988), and intestinal functions (Campbell et al., 1989). However, relatively little is actually known about the functional significance of this binding site.

Some of the confusion surrounding the functional significance of σ binding sites may, in part, stem from the existence of at least two (Gundlach et al., 1986; Hellewell and Bowen, 1990; Vu et al., 1990; Walker et al., 1990; Wolfe et al., 1988, 1989) and possibly three (Meyers et al., 1994), subtypes of σ binding sites. A second σ binding site was first identified in PC12 cells, a cell line derived from the rat adrenal medulla (Bowen and Hellewell, 1988). In this study it was shown that σ site ligands showed affinities for a σ site in a rank order of potency which differed from the profile seen in the guinea pig brain (Bowen and Hellewell, 1988). These sites were later classified as σ_2 binding sites (Hellewell and Bowen, 1990). It was soon shown that σ_1 and σ_2 sites exist in both the brain and periphery in a wide variety of species (Gundlach et al., 1986; Vu et al., 1990; Wolfe et al., 1988, 1989). Although both σ_1 and σ_2 sites possess high affinity for haloperidol and DTG, there are several differences. σ_1 Sites are enan-

* Corresponding author. Tel.: (716) 274-5673; fax: (716) 272-3910; e-mail: KINNEY_G@FISONS.COM.

SSDI 0014-2999(95)00584-6

tioselective for (+)-benzomorphans, whereas σ_2 sites are enantioselective for (−)-benzomorphans (Bowen and Hellewell, 1988; Hellewell and Bowen, 1990). Further, σ_1 and σ_2 sites occur as proteins with different molecular weights (approximately 25 kDa and 18–21 kDa, respectively) (Hellewell and Bowen, 1990). Finally, σ_1 and σ_2 sites have different anatomical distribution among species. For example, there is a higher concentration of σ_2 compared to σ_1 sites in the rat liver than in the guinea pig brain (Hellewell et al., 1990).

Functionally, it has been shown that electrically induced contractions of the guinea pig vas deferens are potentiated by compounds with a high affinity for the σ site, with (−)-opiates acting more potently than (+)-opiates which is indicative of σ_2 site mediation (Vaupel and Su, 1987). However, these results appear at conflict with the results of binding studies using vas deferens tissue, which showed a σ_1-like binding profile (Su and Wu, 1990). Campbell et al. (1989) have shown that σ site ligands decrease contractions of the guinea pig ileum longitudinal muscle/myenteric plexus preparation induced by electrical stimulation or application of 5-hydroxytryptamine agonists via a non-cholinergic mechanism. While binding data were presented in this latter report, no data were presented as to the relative affinities of the compounds tested for the σ_1 versus σ_2 site subtype. Given the clear differences between these two subtypes, they may subserve very different functions physiologically. Thus, the present study examined the effects of a variety of σ site ligands on electrically induced contractions of the guinea pig ileum longitudinal muscle/myenteric plexus preparation. In addition, the relative affinity of these compounds for both σ_1 and σ_2 binding sites was assessed.

2. Materials and methods

2.1. σ Receptor binding assay

Binding assays were performed using a modification of previously described methods (De Costa et al., 1989; Bowen et al., 1988, 1990). Briefly, frozen male Hartley guinea pig (Charles Rivers, MA, USA) brains with the cerebella removed were thawed at room temperature. All procedures were conducted at 4°C unless otherwise noted. The brains were homogenized in ice-cold 0.32 M sucrose in 10 mM Tris-Cl (pH 7.4) with a motor-driven Teflon-glass homogenizer in a volume of 10 ml/g of tissue. The homogenate was centrifuged at $1000 \times g$ for 10 min in a refrigerated centrifuge. The supernatant was removed and the pellet was resuspended in ice-cold 0.32 M sucrose, 10 mM Tris-Cl (pH 7.4, 5 ml/g) and again centrifuged at $1000 \times g$ for 10 min. The supernatants were combined and centrifuged at $31\,000 \times g$ for 15 min. The pellet was resuspended in 10 volumes of 10 mM Tris-Cl (pH 7.4) and allowed to incubate at room temperature for 15 min followed by centrifugation at $31\,000 \times g$ for 15 min. The pellet was then resuspended in 10 volumes of 5 mM Tris-Cl (pH 8.0). Just prior to the assay, the suspension was homogenized for 30 s. The σ_1 assay was carried out in a total volume of 0.5 ml, consisting of 5 mM Tris-Cl (pH 8.0), 1–2 nM [^{3}H](+)-pentazocine (15–30 Ci/mM, Dupont), 0.1 ml of membrane suspension (1.5–2.0 mg protein), and various concentrations of the test compounds.

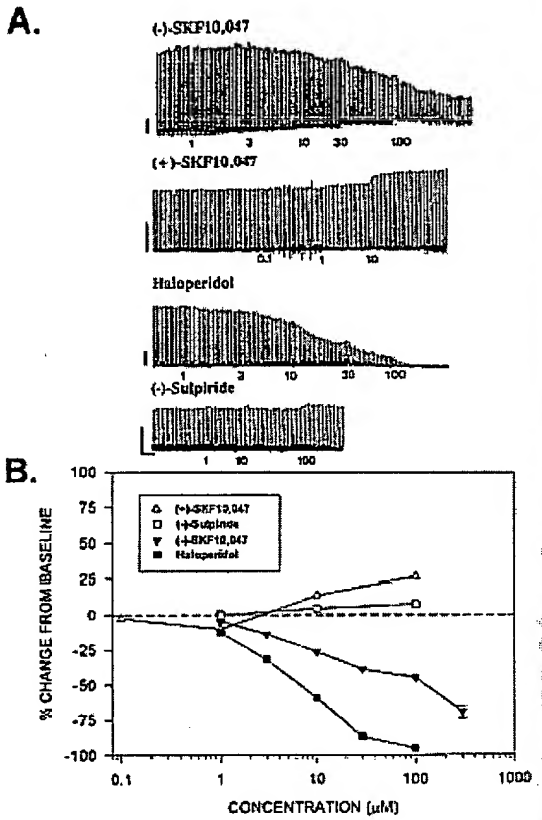


Fig. 1. A: Effects of (−)-SKF10,047, (+)-SKF10,047, haloperidol and (−)-sulpiride, respectively, on electrically evoked contraction of guinea pig ileum longitudinal muscle/myenteric plexus preparation. Compare the concentration-dependent decreases in electrically evoked guinea pig ileum longitudinal muscle/myenteric plexus contractions produced by (−)-SKF10,047 and haloperidol with the lack of effect of (+)-SKF10,047 and (−)-sulpiride. Scale bars: vertical = 0.5 g; horizontal = 1 min. Numbers at the bottom of the tracings represent the concentration (μM) of the given compound. B: Effects of (+)-SKF10,047, (−)-sulpiride, (−)-SKF10,047 and haloperidol on electrically evoked guinea pig ileum longitudinal muscle/myenteric plexus contractions. Error bars represent standard error of the means.

Nonspecific binding was determined by including 100 μM (+)-pentazocine. Specific binding was determined by subtracting the dpm bound in the presence of 100 μM (+)-pentazocine from that bound in the absence of added (+)-pentazocine or test compound. After 16–18 h of incubation at room temperature, the membranes were collected onto a Whatman GF/B filter (Brandell M-48 Harvester), which was presoaked overnight in 0.3% polyethylenimine. The assay tubes and filters were rinsed 2 times with 4–5 ml cold 5 mM Tris-Cl (pH 8.0) and the filters were counted in a scintillation counter after soaking in Ecolume (ICN) scintillation fluid for 16–18 h. Protein was measured with a BCA protein reagent assay kit (Pierce Chemical Co.), using bovine serum albumin as the standard.

Affinity for σ_2 sites was measured in the same way as described for [^{3}H](+)-pentazocine binding, except male Sprague-Dawley rat (Harlan, WI, USA) liver membrane prepared from fresh liver was used as the receptor source, and [^{3}H]DTG (25–30 Ci/mM, Dupont) was used as the ligand. The reaction was carried out in 10 mM Tris-Cl (pH 7.4), with dextromethorphan (5 μM) included to mask σ_1 sites.

IC_{50} values, 95% confidence intervals, and standard error of the means (S.E.M.) were obtained using a nonlinear curve fitting program (Graph Pad). All binding experiments (1–6 experiments per compound) were performed in triplicate for each compound tested.

2.2. *Guinea pig ileum longitudinal muscle / myenteric plexus functional assay*

Sections of guinea pig ileum longitudinal muscle/myenteric plexus were prepared as described by Campbell et al. (1989). Briefly, an 8–10 cm section of small intestine 10–25 cm proximal to the ileocecal junction was removed from male Hartley guinea pigs (200–300 g, Charles Rivers, MA, USA) following killing of the animal by overexposure to CO_2. The removed intestine was immediately placed in a Krebs solution with the following composition (mM): NaCl, 118; KCl, 4.75; $CaCl_2$, 2.54; $MgCl_2$, 1.17; $NaHCO_3$, 25.0; KH_2PO_4, 0.93; dextrose, 11.0. Sections of intestine approximately 1–2 cm in length were gently stretched over a moistened glass rod and a longitudinal cut was made with a scalpel blade. The longitudinal muscle/myenteric plexus was gently removed using a cotton-tip applicator moistened with the Krebs solution. The strips were placed in 20 ml organ baths containing the Krebs solution at 37.0–37.5°C and bubbled with a 5% CO_2 and 95% O_2 gas. The strips were placed under a 1 g load and contractility was measured using Grass FT03 transducers connected to a Gould 2600S chart recorder. The strips were stimulated at 0.05 Hz using a Grass dual channel stimulator (S88) set at maximal voltage (≈ 150 V, 1–10 ms in duration). Platinum concentric electrodes positioned above and below the strips delivered the stimulation current. Following a 90–120 min equilibrium period during which the bath was changed several times, test compounds were administered in a cumulative fashion, allowing a minimum of 5 min before additional compound was added to the bath. The concentration-effect curves of (−)-pentazocine and (−)-SKF10,047 were determined in the presence of 10 μM naloxone due to the affinity of these compounds for μ-opioid receptors. The effectiveness of a given compound to inhibit electrically induced contraction was measured as the percent change from baseline conditions. The concentration of a given test compound to produce a half-maximal inhibition of the electrically induced contraction (IC_{50}) was determined with a nonlinear curve-fit program (Interactive Statistical System) using the mean response of at least 3 separate trials as the given response for a single concentration. The correlations between IC_{50} values to inhibit electrically induced contractions and affinities

Table 1
Efficacy and affinity values for various σ ligands (± S.E.M.)

Compound	σ_1 Site binding IC_{50} (nM)	σ_2 Site binding IC_{50} (nM)	Inhibition of GPLMMP contraction IC_{50} (μM)	[a] Musc. Ach. binding (nM)	[a] PCP binding (nM)	[a] Dopamine D_2 binding (nM)
1. DTG	137.0 ± 5	100.0 ± 1.7	5.267 ± 0.38	3600 [c]	12000 [d]	> 100000 [e]
2. BMY-14802	300.0 ± 22	131.0 ± 13.6	5.282 ± 0.46	32100 [f]	> 200000 [f]	4000 [f]
3. Haloperidol	3.5 ± 0.1	322.7 ± 33	5.687 ± 0.46	2700 [e]	5000 [g]	1.9 [h]
4. (−)-Pentazocine	251.0 ± 1.7	345.0 ± 26	5.98 ± 0.7 [b]	2500 [e]	N.T.	N.T.
5. Rimcazole	7360.0 ± 2830	2740.0 ± 361	6.253 ± 0.48	27000 [f]	> 200000 [f]	86000 [f]
6. (+)-Pentazocine	3.4 ± 0.05	4077.5 ± 155	16.748 ± 0.68	800.0 [e]	1900 [i]	> 100000 [i]
7. (−)-SKF10,047	8060.0 ± 3280	7763.0 ± 150	19.209 ± 4.8 [b]	N.T.	5650 [j]	14000 [h]
8. Dextromethorphan	577.3 ± 5.4	14300 ± 1180	42.158 ± 0.53	N.T.	2500 [j]	N.T.
9. (+)-SKF10,047	218.0 ± 13.5	54633 ± 2300	> 100	N.T.	1170 [j]	29000 [h]
10. (−)-Sulpiride	> 1000000	> 1000000	> 100	87000 [e]	N.T.	301.0 [h]

[a] K_i or IC_{50}. [b] Tested in the presence of 10 μM naloxone. [c] Bowen et al. (1992). [d] Contreras et al. (1988). [e] Coccini et al. (1991). [f] Largent et al. (1988). [g] Meltzer et al. (1992). [h] Tam and Cook (1984). [i] Iyengar et al. (1990). [j] Murray and Leid (1984). N.T. = not tested or not found in literature.

for binding sites were assessed using a rank correlation test and/or the Pearson product-moment correlation test.

2.3. Drugs

(+)-*N*-Allylnormetazocine ((+)-SKF10,047), (−)-*N*-allylnormetazocine ((−)-SKF10,047) and rimcazole · HCl were obtained from Research Biochemicals International, Natick, MA, USA. Haloperidol, 1,3-di-*o*-tolylguanidine (DTG) and (−)-sulpiride were obtained from Sigma Chemical Co., St. Louis, MO, USA. (+)-Pentazocine and (−)-pentazocine were synthesized in the Chemistry Department of Fisons Pharmaceuticals (now Astra Arcus USA), Rochester, NY, USA. Dextromethorphan and (±)-α-(4-fluorophenyl)-4-(5-fluoro-2-pyrimidinyl)-1-piperazine-butanol (BMY-14802) were generous gifts from Roche Chemicals and Bristol-Myers Squibb, respectively. With the exception of DTG, all compounds were dissolved in sterile H_2O and delivered at the concentrations indicated. DTG was first dissolved in a minimal amount of dilute lactic acid, brought to a pH of 7.0 using dilute NaOH, and brought to volume with H_2O.

3. Results

With the exception of (+)-SKF10,047 and (−)-sulpiride, all of the compounds tested produced concentration-dependent inhibition of electrically induced guinea pig ileum longitudinal muscle/myenteric plexus contractions (Table 1 and Fig. 1). The most potent compounds (DTG, BMY-14802, haloperidol and (−)-pentazocine) were compounds with high affinity for the σ_2 binding site (IC_{50} values = 0.1–0.345 μM), but not necessarily high selectively over the σ_1 site. The ratios of σ_2/σ_1 binding for DTG, BMY-14802, haloperidol and (−)-pentazocine were 0.73, 0.44, 92.2 and 1.38, respectively. The rank order of compounds to inhibit

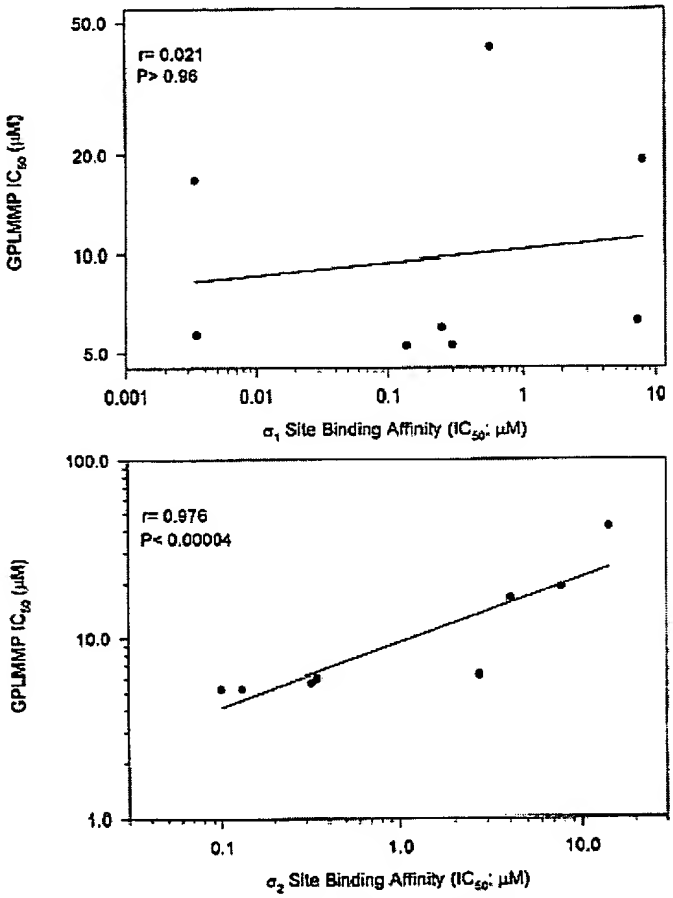


Fig. 2. Correlation of binding affinities for the σ_1 (top panel) and σ_2 (bottom panel) binding sites and efficacy in the guinea pig longitudinal muscle/myenteric plexus preparation assay (GPLMMP) for tested compounds (see Table 1).

guinea pig ileum longitudinal muscle/myenteric plexus contractions was perfectly correlated with their rank affinities for the σ_2 ($r = 1.0$), but not the σ_1 ($r = 0.283$, $P > 0.46$) binding site. Additional analysis, using previously reported binding affinities (Table 1), revealed no significant correlations between rank efficacy to inhibit guinea pig ileum longitudinal muscle/myenteric plexus contractions and rank affinity for muscarinic acetylcholine ($r = 0.036$, $P > 0.939$), D_2 dopamine ($r = -0.181$, $P > 0.669$), or MK-801 ($r = -0.577$, $P > 0.175$) binding sites.

Fig. 2 compares the correlations between binding affinity for σ_1 (top panel) or σ_2 (lower panel) sites and activity in the guinea pig ileum longitudinal muscle/myenteric plexus assay. As seen in Fig. 2 and quantified using the Pearson product-moment correlation, there is no significant relationship between σ_1 site binding and guinea pig ileum longitudinal muscle/myenteric plexus activity ($r = 0.021$, $P > 0.96$), whereas this relationship for σ_2 site binding and guinea pig ileum longitudinal muscle/myenteric plexus activity is extremely significant ($r = 0.976$, $P < 0.000034$).

4. Discussion

The results show that the efficacy of σ site ligands to inhibit electrically induced contractions of the guinea pig ileum is highly correlated with affinity for the σ_2, but not σ_1 site subtype. The lack of correlation to previously reported muscarinic acetylcholine receptor binding indicates that the effects of the σ site ligands tested were neither due to non-selective blockade of receptors on smooth muscle nor activation of muscarinic receptors on myenteric cholinergic neurons. This latter finding is in agreement with Campbell et al. (1989), in which a variety of σ binding site ligands produced no alterations on the inhibitory effect of bethanechol in this preparation. The lack of inhibition by (−)-sulpiride and MK-801 (data not shown) further underscores the lack of involvement of dopamine and MK-801 receptor sites, respectively, in this assay. Also in agreement with Campbell et al. (1989), we found that (+)-SKF10,047 occasionally produced an increase in electrically induced contractility. The mechanism responsible for this increase remains unclear.

To our knowledge this is the first demonstration of a functional in vitro assay with the ability to selectively identify actions at the σ_2 binding site, providing a starting point from which to assess the functional characteristics of this system. As such, these data have vast implications in future research into the etiology of numerous disorders which have been linked to σ binding sites. In addition, these results represent the first evidence that guinea pig intestinal motility is specifically modulated by a σ_2 mechanism.

These studies further indicate that caution should be used when attempting to assign a σ based mechanism to a functional assay. For example, the choice of [^{3}H]DTG or [^{3}H]haloperidol for affinity studies may be less than ideal since both bind with relatively high affinity to both σ_1 and σ_2 sites. Thus, any conclusions based on such binding studies, which attempt to link σ site ligands to biological function, may prove inadequate. This is illustrated in that there are no significant correlations (using Pearson product-moment correlational analyses) between the efficacy of the compounds tested in the present study to inhibit guinea pig ileum longitudinal muscle/myenteric plexus contraction and the K_i values of these compounds to displace [^{3}H]DTG, [^{3}H]pentazocine, [^{3}H](+)-PPP or [^{3}H](+)-SKF10,047 in the guinea pig brain ($P > 0.88$, $P > 0.72$, $P > 0.88$, $P > 0.82$, respectively), as reported in previous studies (see Walker et al., 1990 for review). However, when σ_1 and σ_2 binding was differentially determined, as in the present study, the efficacy of these compounds is strongly correlated with affinity at the σ_2 subtype. Thus, it appears that additional analysis of past studies may reveal specific modulation of functional systems by specific σ subtypes. In the only previous study to examine the relationship between σ ligand binding and guinea pig ileum longitudinal muscle/myenteric plexus efficacy (Campbell et al., 1989), a very low overall correlation was found (using least squares linear regression) between displacement of [^{3}H]DTG in the guinea pig brain and the efficacy of all tested compounds to inhibit electrically stimulated guinea pig ileum longitudinal muscle/myenteric plexus contractions ($r = 0.37$) (Campbell et al., 1989; Walker et al., 1990). This low correlation may have reflected the fact that modulation of the guinea pig ileum longitudinal muscle/myenteric plexus bioassay is linked to a compound's affinity for the σ_2 binding site, and not the σ_1 binding site. As such, the development of new functional assays will require careful examination of the affinity of test compounds for specific σ site subtypes. Development of such σ subtype specific assays is crucial for forwarding investigations into the etiology of disease states in which σ sites have been implicated.

One such condition, as indicated by the present results, is irritable bowel syndrome. Irritable bowel syndrome is characterized by an increase in tone and spasticity of the gastrointestinal tract, most notably the lower bowel (Thompson, 1993). Antimuscarinic compounds (e.g., belladonna alkaloids) are often prescribed for treatment of irritable bowel syndrome; however, the effective dose range for these compounds is near the maximally tolerated dose resulting in side effects typically associated with anticholinergic treatment. As a result patient compliance in long-term treatment is often poor. Thus, alternative approaches for treatment of this condition, and other irritative

conditions of the bowel (e.g., irritable colon, spastic colon, mucous colitis, acute enterocolitis, diverticulitis, and dysenteries), should focus on non-anticholinergic mechanisms for treatment. The present evidence for a role of the σ_2 site in the mediation of ileal function suggests a novel therapeutic target which may not suffer from the accompaniment of the multitude of side-effects associated with anticholinergic compounds.

In summary, the present results demonstrate the first bioassay sensitive to compounds with affinity for σ_2 sites, providing the first means by which elucidation into the functional role of σ_2 sites can be achieved. Further, the results demonstrate the necessity to take into account the subtype specificity of σ site ligands when using them as investigative tools for research into the numerous disorders in which σ related processes have been implicated to play a role. Finally, these results demonstrate an immediate application with regard to irritable bowel syndrome.

Acknowledgements

The authors wish to thank Drs. Roy D. Simmons, Jerry A. Miller and Rae R. Matsumoto for their helpful discussions and comments during the preparation of this paper and Dr. Miller for his help with the construction of Fig. 1.

References

Bowen, W.D. and S.B. Hellewell, 1988, Characterization of sigma receptors on PC12 cells: pharmacological differences from rat and guinea pig brain indicate sigma receptor heterogeneity, Soc. Neurosci. Abstr. 14, 703.

Bowen, W.D., B.N. Kirschner, A.H. Newman and K.C. Rice, 1988, σ Receptors negatively modulate agonist-stimulated phosphoinositide metabolism in rat brain, Eur. J. Pharmacol. 149, 399.

Bowen, W.D., E.L. Moses, P.J. Tolentino and J.M. Walker, 1990, Metabolites of haloperidol display preferential activity at sigma receptors compared to dopamine D-2 receptors, Eur. J. Pharmacol. 177, 111.

Bowen, W.D., P.J. Tolentino, K.K. Hsu, J.M. Cutts and S.S. Naidu, 1992, Inhibition of the cholinergic phosphoinositide response by sigma ligands: distinguishing a sigma receptor-mediated mechanism from a mechanism involving direct cholinergic receptor antagonism, in: Multiple Sigma and PCP Receptor Ligands: Mechanisms for Neuromodulation and Neuroprotection?, eds. J. Kamenka and E.F. Domino (NPP Books, Ann Arbor, MI) p. 155.

Campbell, B.G., M.W. Scherz, J.F.W. Keana and E. Weber, 1989, Sigma receptors inhibit contractions of the guinea pig ileum longitudinal muscle/myenteric plexus preparation elicited by both electrical stimulation and exogenous serotonin, J. Neurosci. 9, 3380.

Coccini, T., L. Manzo and L.G. Costa, 1991, Spiperone labels sigma receptors, not dopamine D_2 receptors, in rat and human lymphocytes, Immunopharmacology 22, 93.

Contreras, P.C., R. Quirion, D.R. Gehlert, M.L. Contreras and T.L. O'Donohue, 1987, Autoradiographic distribution of non-dopaminergic sigma binding sites labeled by [^{3}H]haloperidol in rat brain, Neurosci. Lett. 75, 133.

Contreras, P.C., M.L. Contreras, T.L. O'Donohue and C.C. Lair, 1988, Biochemical and behavioral effects of sigma and PCP ligands, Synapse 2, 240.

De Costa, B.R., W.D. Bowen, S.B. Hellewell, J.M. Walker, A. Thurkauf, A.E. Jacobson and K.C. Rice, 1989, Synthesis and evaluation of optically pure [^{3}H]-(+)-pentazocine, a highly potent and selective radioligand for σ receptors, FEBS Lett. 251, 53.

Gundlach, A.L., B.L. Largent and S.H. Snyder, 1986, Autoradiographic localization of σ-receptor binding sites in guinea pig and rat central nervous system with (+)-^{3}H-3-(3-hydroxyphenyl)-*N*-(1-propyl)-piperidine, J. Neurosci. 6, 1757.

Hellewell, S.B. and W.D. Bowen, 1990, A sigma-like binding site in rat pheochromocytoma (PC12) cells: decreased affinity for (+)-benzomorphans and lower molecular weight suggest a different sigma receptor form from that of the guinea pig brain, Brain Res. 527, 244.

Hellewell, S.B., A.E. Bruce and W.D. Bowen, 1990, Characterization of 'sigma-like' binding sites in rat liver membranes: further evidence for sigma-1 and sigma-2 sites, in: New Leads in Opioid Research: Proceedings of the International Narcotics Research Conference, International Congress Series 914, eds. J.M. Van Ree, A.H. Mulder, V.M. Wiegant and T.B. Van Wimersma Greidanus (Excerpta Medica – Elsevier, Amsterdam) p. 270.

Hudzik, T.J., 1991, Sigma ligand-induced emesis in the pigeon, Pharmacol. Biochem. Behav. 41, 215.

Iyengar, S., V.M. Dilworth, S.J. Mick, P.C. Contreras, J.B. Monahan, T.S. Rao and P.L. Wood, 1990, Sigma receptors modulate both A9 and A10 dopaminergic neurons in the rat brain: functional interaction with NMDA receptors, Brain Res. 524, 322.

Largent, B.L., H. Wikstrom, A.M. Snowman and S.H. Snyder, 1988, Novel antipsychotic drugs share high affinity for σ receptors, Eur. J. Pharmacol. 155, 345.

Martin, W.R., C.E. Eades, J.A. Thompson and R.E. Huppler, 1976, The effects of morphine- and nalorphine-like drugs in the nondependent and morphine-dependent chronic spinal dog, J. Pharmacol. Exp. Ther. 197, 517.

Matsumoto, R.R., W.D. Bowen and J.M. Walker, 1989, Age-related differences in the sensitivity of rats to a selective sigma ligand, Brain Res. 504, 145.

Matsumoto, R.R., M.K. Hemstreet, N.L. Lai, A. Thurkauf, B.R. De Costa, K.C. Rice, S.B. Hellewell, W.D. Bowen and J.M. Walker, 1990, Drug specificity of pharmacological dystonia, Pharmacol. Biochem. Behav. 36, 151.

Meltzer, L.T., C.L. Christoffersen, K.A. Serpa, T.A. Pugsley, A. Razmpour and T.G. Heffner, 1992, Lack of involvement of haloperidol-sensitive sigma binding sites in modulation of dopamine neuronal activity and induction of dystonias by antipsychotic drugs, Neuropharmacology 31, 961.

Meyers, A.M., P.S. Charifson, C.E. Owens, N.S. Kula, A.T. McPhail, R.J. Baldessarini, R.G. Booth and S.D. Wyrick, 1994, Conformational analysis, pharmacophore identification, and comparative molecular field analysis of ligands for the neuromodulatory σ_1 receptor, J. Med. Chem. 37, 4109.

Murray, T.F. and M.E. Leid, 1984, Interaction of dextrorotatory opioids with phencyclidine recognition sites in rat brain membranes, Life Sci. 34, 1899.

Musacchio, J.M., M. Klein and L.J. Santiago, 1988, High affinity dextromethorphan binding sites in guinea pig brain: further characterization and allosteric interactions, J. Pharmacol. Exp. Ther. 247, 424.

Su, T.P., 1982, Evidence for sigma opioid receptor: binding of [^{3}H]SKF-10047 to etorphine-inaccessible sites in guinea-pig brain, J. Pharmacol. Exp. Ther. 223, 284.

Su, T.P., 1993, Delineating biochemical and functional properties of sigma receptors: emerging concepts, Crit. Rev. Neurobiol. 7, 187.
Su, T.P. and X.Z. Wu, 1990, Guinea pig vas deferens contains sigma but not phencyclidine receptors. Neurosci. Lett. 108, 341.
Su, T.P., S.E. Schell, F.Y. Ford-Rice and E.D. London, 1988, Correlation of inhibitory potencies of putative antagonists for σ receptors in brain and spleen, Eur. J. Pharmacol. 148, 467.
Tam, S.W. and L. Cook, 1984, Sigma opiates and certain antipsychotic drugs mutually inhibit (+)-[^{3}H]SKF10,047 and [^{3}H]haloperidol binding in guinea pig brain membranes, Proc. Natl. Acad. Sci. USA 81, 5618.
Thompson, W.G., 1993, Irritable bowel syndrome: pathogenesis and management, Lancet 341, 1569.
Vaupel, D.B. and T.P. Su, 1987, Guinea-pig vas deferens preparation may contain both σ receptors and phencyclidine receptors, Eur. J. Pharmacol. 139, 125.
Vu, T.H., A.D. Weissman and E.D. London, 1990, Pharmacological characteristics and distributions of sigma and phencyclidine receptors in the animal kingdom, J. Neurochem. 54, 598.
Walker, J.M., W.D. Bowen, F.O. Walker, R.R. Matsumoto, B. De Costa and K.C. Rice, 1990, Sigma receptors: biology and function, Pharmacol. Rev. 42, 355.
Weber, E., M. Sonders, M. Quarum, S. McLean, S. Pou and J.F.W. Keana, 1986, 1,3-di(2-[5-^{3}H]tolyl)guanidine: a selective ligand that labels σ-type receptors for psychotomimetic opiates and antipsychotic drugs, Proc. Natl. Acad. Sci. USA 83, 8784.
Wolfe Jr., S.A., C. Kulsakdinun, G. Battaglia, J.H. Jaffe and E.B. De Souza, 1988, Initial identification and characterization of sigma receptors on human peripheral blood leukocytes, J. Pharmacol. Exp. Ther. 247, 1114.
Wolfe, S.A., S.G. Culp and E.B. De Souza, 1989, σ-Receptors in the endocrine organs: identification, characterization, and autoradiographic localization in rat pituitary, adrenal, testis, and ovary, Endocrinology 124, 1160.

σ Ligands with Subnanomolar Affinity and Preference for the σ_2 Binding Site. 1. 3-(ω-Aminoalkyl)-1*H*-indoles

Jens Perregaard,* Ejner K. Moltzen, Eddi Meier, and Connie Sánchez

Research and Development, H. Lundbeck A/S, Ottiliavej 9, DK-2500 Copenhagen-Valby, Denmark

Received December 27, 1994[⊗]

A series of 4-(1*H*-indol-3-yl)-1-butyl-substituted 4-phenylpiperidines, 4-phenyl-1,2,3,6-tetrahydropyridines, and 4-phenylpiperazines was synthesized. The phenyl group was optionally substituted with 4-fluoro or 2-methoxy substituents. High affinity for both σ_1 and σ_2 binding sites was achieved with these compounds. Additionally, these compounds had relatively high affinity for serotonin 5-HT_{1A} and 5-HT_{2A}, dopamine D_2, and adrenergic α_1 receptors. Introduction of a 4-fluorophenyl substituent at the indole nitrogen atom rendered very selective σ_2 ligands with subnanomolar affinity for the σ_2 binding site. The prototype of such a compound was 1-(4-fluorophenyl)-3-[4-[4-(4-fluorophenyl)-1-piperidinyl]-1-butyl]-1*H*-indole, **11a** (code no. Lu 29-253). This compound had the following binding affinities: IC_{50} (σ_1) = 16 nM, IC_{50} (σ_2) = 0.27 nM, IC_{50} (5-HT_{1A}) = 22 000 nM, IC_{50} (5-HT_{2A}) = 270 nM, IC_{50} (D_2) = 4200 nM, IC_{50} (α_1) = 220 nM. Spiro-joining of the phenyl and the piperidine rings into a spiro[isobenzofuran-1(3*H*),4′-piperidine] ring system resulted in even more selective compounds. Variations of the 1-substituent at the indole and of the chain length of the alkylene spacer group were studied. The optimal compound was the spiro analogue of compound **11a**. This compound is 1′-[4-[1-(4-fluorophenyl)-1*H*-indol-3-yl]-1-butyl]spiro[isobenzofuran-1(3*H*),4′-piperidine], **14f** (code no. Lu 28-179), with the binding affinities: IC_{50} (σ_1) = 17 nM, IC_{50} (σ_2) = 0.12 nM, IC_{50} (5-HT_{1A}) = 21 000 nM, IC_{50} (5-HT_{2A}) = 2000 nM, IC_{50} (D_2) = 800 nM, IC_{50} (α_1) = 330 nM. However, the most selective σ_2 versus σ_1 ligand was the tropane derivative 1-(4-fluorophenyl)-3-[4-[3-(4-fluorophenyl)-8-azabicyclo[3.2.1]oct-2-en-8-yl]-1-butyl]-1*H*-indole, **15a**. This compound had the following binding affinitites: IC_{50} (σ_1) = 1200 nM, IC_{50} (σ_2) = 2.5 nM. Potent anxiolytic activity in the black/white box exploration test in rats was found with the two most prominent σ_2 ligands Lu 29-253 and Lu 28-179. Good penetration into the CNS was documented both after subcutaneous and peroral administration of Lu 28-179 by ex vivo binding studies. Long duration of action was demonstrated both in ex vivo binding ($T_{1/2}$ ~ 20 h) and in the black/white box exploration test.

Introduction

Focus on development of very potent and selective σ ligands has been intensified during recent years. Recognition of σ binding sites was originally based on the finding that benzomorphans, such as SK&F 10,047 and pentazocine, had additional binding to non-opiate receptors[1,2] and on the clinical observation of psychomimetic side effects of these compounds.[3] The existence of at least two distinct σ binding sites denoted σ_1 and σ_2 has recently become evident.[4,5] The (+)-enantiomers of opiate ligands, such as (+)-pentazocine (**1**), specifically label the σ_1 binding site, while, until now, no highly selective σ_2 ligand has been available. (−)-Pentazocine is a mixed σ_1/σ_2 ligand of moderate affinity. Efforts have mainly been addressed to the design and synthesis of selective σ_1 ligands, although some attempts to elucidate the structural elements that determine σ_2 preference were reported.[6,7] Quite recently, some (+)-isomers of certain 5-phenylmorphane derivatives were reported to exhibit high affinity and selectivity for the σ_2 binding site. However, these compounds also seem to exhibit high affinity for opiate μ receptors.[8] It must be emphasized that interpretation of σ_1/σ_2 selectivity from results dated more than 2−3 years back is often quite confusing. Binding assays at that time were generally nonselective, both regarding radioligands and the biological tissue applied. As the characterization of the subtypes of binding sites generally only involves ligand binding with no indication of functionality, it has been suggested that the term receptors should be avoided.[4] The role of the different σ binding sites in psychiatric conditions still remains speculative. At a very early stage rimcazole (**2**), which is a weak but rather selective σ ligand,[9] was tested in the clinic in schizophrenic patients. Though some limited but promising results from these early phase II studies have been published,[10,11] the clinical development was discontinued by Burroughs-Wellcome. (+)-Pentazocine was reported to have anxiogenic properties while the opposite enantiomer induced relaxation in humans.[12] In rats both (+)-pentazocine and 1,3-di(2-tolyl)guanidine (DTG) were anxiogenic.[13] Newer and more potent, but also unselective, σ ligands, such as BMY 14802 (**3**)[14] and DuP 734 (**4**),[15,16] were predicted to have antipsychotic potential from animal experiments. Both of these compounds have proceeded to clinical trials.

Some years ago, during the development of low-efficacy serotonin 5-HT_{1A} agonists,[17,18] we found that within a series of 1-[4-(1*H*-indol-3-yl)-1-butyl]-4-arylpiperazines and corresponding indoline derivatives **7** some of the indole derivatives were quite potent σ ligands as well. We became interested in exploring the possibilities of developing selective high-affinity σ ligands based on this series of compounds. The antipsychotic butyrophenone derivative haloperidol (**5**) is a potent σ ligand

[⊗] Abstract published in *Advance ACS Abstracts*, May 1, 1995.

0022-2623/95/1838-1998$09.00/0

Table 1. Structures and σ Binding Affinities of 4-Phenylpiperidines and 1-Phenylpiperazines[a]

compd	X	Y	σ binding affinities[b] (IC_{50} values, nM) [^{3}H]-(+)-pentazocine (σ_1)	[^{3}H]DTG (σ_2)	σ_1/σ_2
9a	N	2-OCH_3	14.	21.	0.67
9b	CH	2-OCH_3	4.5	3.3	1.4
9c	N	4-F	5.6	1.3	4.3
9d	CH	H	1.5	0.48	3.1
9e	CH	4-F	1.4	4.0	0.35
9f	C—	4-F	7.5	2.3	3.3
11a	CH	4-F	16.	0.27	59.
11b	N	4-F	440.	7.5	59.
11c	CH	H	44.	0.44	100.
11d	N	H	27.	0.69	39.
15a[c]			1200.	2.5	480.
DTG			36.	52.	0.69
1 (+)-pentazocine			3.0	2100.	0.0014
(−)-pentazocine			8.9	29.	0.31
2 rimcazole			690.	180.	3.8
3 BMY 14802			60.	230.	0.26
4 DuP 734			2.6	23.	0.11
5 haloperidol			0.65	17.	0.038
6 L-687,384			0.26	12.	0.021

[a] Structures 9 and 11 refer to Scheme 1. Structures of reference compounds are shown in Figure 1. [b] Results are expressed as IC_{50} values (nM) and are the logarithmic mean of at least two, or, in the case of the σ_2 binding, three determinations. Two full (in the case of σ_2, three full) concentration curves were measured using five concentrations of test drug in triplicate (covering three decades). Sd ratios were obtained by calculating the variance of repeated measures of ratios between the first and second IC_{50} determination for a series of n drugs. In cases of ratios greater than 2 × sd (95% confidence interval), extra determinations were performed and outliers were discarded. The following sd ratios were obtained: σ_1 1.8 ($n = 74$); σ_2 2.3 ($n = 100$). [c] See Chart 1 for structure.

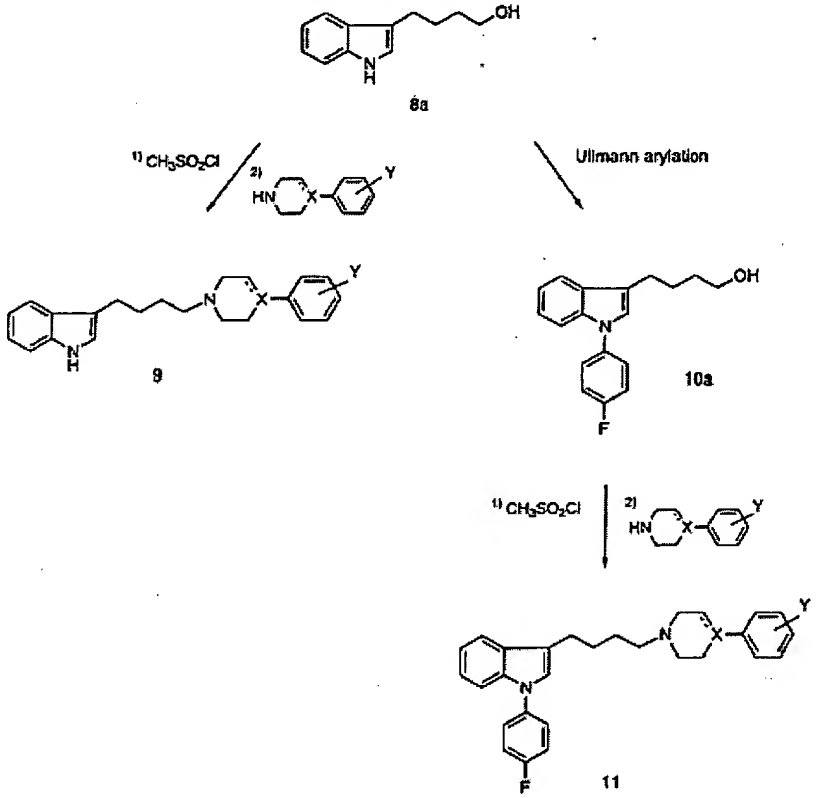
with preference for σ_1 binding sites. However, 5 also blocks classical receptors, especially dopamine D_2 receptors. Many attempts have been made recently to extract the σ pharmacophore of 5 in close analogues, and thus retain σ binding and eliminate interaction with dopamine receptors.[19-22] Compound 3 is actually an example of a haloperidol derivative that has lost affinity for D_2 receptors. Also in our original series of 4-(1*H*-indol-3-yl)-1-butylpiperazines[17,18] with 5-HT_{1A} serotonergic activity, some structural features, such as C-4, chain length, and terminal aryl groups, are common to 5. As the 1-(2-methoxyphenyl)piperazine moiety is a well-established 5-HT_{1A} pharmacophore, it would be interesting to remove the 2-alkoxy substituent and to replace the piperazine ring with a piperidine ring as in 5. In this report and in the succeeding part 2, we present further structural investigations. In this paper substituents at the indole nitrogen atom, spiro-joining of the piperidine and the phenyl rings to a spiro[isobenzofuran-1(3*H*),4'-piperidine] ring system, and variation of length of the interspacing alkylene chain are studied. In part 2 the spiropiperidine system as well as substituents in the benzene part of these sytems are varied, and replacement of the indoloalkyl side chain with more simple alkyl or phenylalkyl side chains is studied. The purpose of this project was to develop selective σ ligands, especially aiming at σ_2 selectivity.

Chemistry

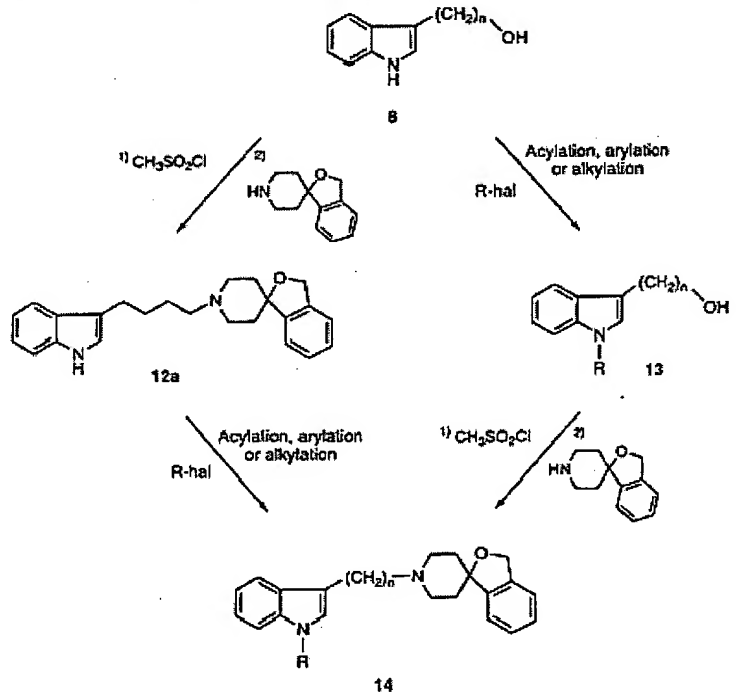
The synthesis of 3-[4-(4-phenyl-1-piperidinyl)-1-butyl]-1*H*-indole, 9d, and 3-[4-[4-(4-fluorophenyl)-1-(1,2,3,6-tetrahydro)pyridinyl]-1-butyl]-1*H*-indole, 9f (Table 1), has been reported by Böttcher et al. in a study of dopaminergic activity of 3-(1,2,3,6-tetrahydropyridylalkyl)indoles.[23] Commercially available 4-(1*H*-indol-3-yl)butanoic acid was coupled with N-unsubstituted 4-phenylpiperidines or 4-phenyl-1,2,3,6-tetrahydropyridines to the corresponding carboxamides by use of *N,N'*-carbonyldiimidazole as coupling agent. The amides were subsequently reduced with dihydrobis(methoxyethanato-*O,O'*)aluminate sodium. We have chosen a slightly different approach for the synthesis in order to minimize the number of synthetic steps after a suitable common intermediate. The methanesulfonate ester of 4-(1*H*-indol-3-yl)-1-butanol (Scheme 1) was prepared in large quantities and high yields by using a modified literature procedure of the synthesis of 4-(1*H*-indol-3-yl)-1-butanol, 8a.[24] Properly substituted phenylpiperidines, phenyl-1,2,3,6-tetrahydropyridines, and phenylpiperazines were easily alkylated with the methanesulfonate ester to 1-unsubstituted indoles, 9. The 1-(4-fluorophenyl)-substituted indoles, 11, were prepared via Ullmann arylation of 4-(1*H*-indol-3-yl)-1-butanol, 8a, with 4-fluoroiodobenzene to give 4-[1-(4-fluorophenyl)-1*H*-indol-3-yl]-1-butanol, 10a. N-Alkylation of piperidines and piperazines was performed using as previously the methanesulfonate ester as a leaving group (Scheme 1). Alternatively, it is also possible to arylate indoles of structure 9 using the Ullmann procedure. A similar sequence is shown in Scheme 2 for the synthesis of spiro derivatives, 14a−14m. Substituents Y in the phenyl ring and the constituents X of the basic 6-membered ring are indicated in Table 1.

In an early paper by Manallack et al.,[25] a planar σ pharmacophore was constructed with the basic nitrogen atom almost in the plane of a benzene ring with its center 5.06 Å away and with the nitrogen electron lone pair almost perpendicular to this plane. At the other end of the receptor, lipophilic substituents at the basic nitrogen atom implicated the presence of a lipophilic pocket as a secondary binding site. A contemporary 5-HT_{1A} receptor model[26,27] had a very similar arrangement of a benzene ring and a basic nitrogen atom 5.2−5.6 Å away from the center of the benzene ring and with the electron lone-pair pointing away from the plane of the benzene ring. These very similar models are a good rationale for the associated σ affinity of our series 7 of 5-HT_{1A} agonists. We wanted to challenge this model and force the benzene and the piperidine rings out of coplanarity in order to position the nitrogen electron

Scheme 1. Synthesis of 1-Unsubstituted (**9**) and 1-(4-Fluorophenyl)-Substituted (**11**) 3-[4-(4-Phenylpiperidin-1-yl)-1-butyl]-1*H*-indoles and Corresponding 1,2,3,6-Tetrahydropyridinyl and Piperazinyl Derivatives



Scheme 2. Synthesis of Spiro[isobenzofuran-1(3*H*),4'-piperidines] with 1-Unsubstituted (**12a**) and 1-Substituted ω-Alkyl-3-indole (**14**) Substituents at the Piperidine Nitrogen Atom



lone pair in the plane of the benzene ring. One way of introducing such conformational restrictions on the benzene and the piperidine rings would be via a spiro-joined system such as the spiro[isobenzofuran-1(3*H*),4'-piperidine] in which the two rings are perpendicular to each other. The unsubstituted spiro-joined piperidine

Table 2. Structures and σ Binding Affinities of Spiro[isobenzofuran-1(3*H*),4′-piperidines] 12a and 14[a]

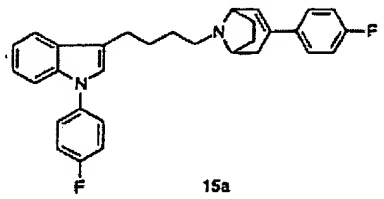
compd	*n*	R	σ binding affinities[b] (IC_{50} values, nM) [^{3}H]-(+)-pentazocine (σ_1)	[^{3}H]DTG (σ_2)	σ_1/σ_2
12a	4	H	2.5	0.41	6.1
14a	4	CH_3	2.1	0.30	7.0
14b	4	CH_3CO	5.7	0.21	27.
14c	4	CH_3SO_2	1.3	0.05	26.
14d	4	$CH_3C_6H_4SO_2$	10.	0.17	59.
14e	4	$C_6H_5CH_2$	5.2	0.48	11.
14f	4	4-F-C_6H_4	17.	0.12	140.
14g	3	4-F-C_6H_4	10.	2.8	3.6
14h	2	4-F-C_6H_4	15.	3.0	5.0
14i	1	4-F-C_6H_4	3.0	4.7	0.64
14j	4	2-thienyl	7.7	0.24	32.
14k	4	3-thienyl	7.0	0.25	28.
14l	4	2-thiazolyl	14.	0.19	74.
14m	4	4-pyridyl	14.	0.41	34.

[a] Structures refer to Scheme 2. [b] See footnote to Table 1.

had previously been synthesized by Marxer et al.[28] Alkylation of this spiropiperidine with the methanesulfonate ester of properly substituted 1*H*-indol-3-yl-ω-alkanols **8** or **13** is shown in Scheme 2. In the butyl series the effect of various substituents R at the indole nitrogen atom was studied. The majority of these substituents were most conveniently introduced on the common intermediate **12a** by acylation or Ullmann arylation procedures. Acylations were performed in the presence of tetrabutylammonium hydrogen sulphate as a phase-transfer catalyst. Heteroaryl substituents in compounds **14j**–**14m** were all introduced by arylating the 1-unsubstituted indole **12a** with the proper bromo heteroaromatic compound. As above, the butanol derivative **10a** was conveniently used to introduce the 4-[1-(4-fluorophenyl)-1*H*-indol-3-yl]-1-butyl substituent in compound **14f**. The 1-alkyl groups (methyl and benzyl) of the indoles **14a** and **14e** were introduced at an early stage in the syntheses by alkylation of potassium salts of 4-(1*H*-indol-3-yl)butanoic acid and 4-(1*H*-indol-3-yl)-1-butanol, respectively. Potassium *tert*-butoxide in DMF was used as base to generate the indole potassium salts. Variation of chain length of the alkylene spacer group from C-1 to C-4 was also studied. The 1-(4-fluorophenyl)-substituted derivatives were chosen for this study. The C-2 and C-3 ($n = 2$ and 3 in Scheme 2) derivatives **14h** and **14g** were prepared from 2-(1*H*-indol-3-yl)-1-ethanol and 3-(1*H*-indol-3-yl)-1-propanol, respectively. These procedures were analogous to the synthesis of the C-4 derivative **14f**. The preparation of the starting 3-indolo-ω-alcohols has previously been described.[24,29] We were not able to synthesize the methanesulfonate ester of 1-(4-fluorophenyl)-1*H*-indol-3-ylmethanol in order to obtain the C-1 derivative. As an alternative method indole-3-carboxaldehyde was arylated to give 1-(4-fluorophenyl)-1*H*-indole-3-carboxaldehyde. Reductive alkylation of spiro[isobenzofuran-1(3*H*),4′-piperidine] with this aldehyde in the presence of sodium cyanoborohydride and molecular sieves afforded the C-1 derivative **14i** in 28% yield. The substituents R at the indole nitrogen atom and the chain length *n* of the alkylene spacer group of the spiropiperidines are indicated in Table 2.

In order to investigate the influence of steric hindrance around the basic piperidine nitrogen atom, we synthesized the tropane analogue **15a** (Chart 1) of the

Chart 1. Structure of the Tropane Derivative 15a



15a

4-(4-fluorophenyl)piperidine derivative **11a**. The 3-(4-fluorophenyl)-8-azabicyclo[3.2.1]oct-2-ene was prepared from 8-methyl-8-azabicyclo[3.2.1]octan-3-one by addition of (4-fluorophenyl)lithium, elimination of water, and removal of the 8-methyl group via carbamate formation. The corresponding procedure is described in detail in the Experimental Section for the synthesis of 4-(4-fluorophenyl)-1,2,3,6-tetrahydropyridine. Alkylation of 3-(4-fluorophenyl)-8-azabicyclo[3.2.1]oct-2-ene with the methanesulfonate ester of 4-[1-(4-fluorophenyl)-1*H*-indol-3-yl]-1-butanol proceeded smoothly to compound **15a**.

Results and Discussion

The pharmacological test models are described in detail in the Experimental Section. Binding affinities for the σ_1 and the σ_2 binding sites are reported in Tables 1 and 2 and compared to relevant reference compounds (structures, see Figure 1). [^{3}H]-(+)-Pentazocine was used as ligand for labeling σ_1 binding sites. [^{3}H]DTG labeling of whole rat brain homogenates, except cerebellum, was used to determine σ_2 binding affinities. It has been concluded that this assay is specific for determination of σ_2 binding affinities.[4,30] (+)-Pentazocine had only insignificant affinity (Table 1) and for a series of compounds identical binding data were obtained both with and without the presence of an excess of (+)-pentazocine in our [^{3}H]DTG binding assay. These data strongly support the σ_2 selectivity of our assay. Table 3 shows binding data for selected compounds to 5-HT_{1A} receptors and other receptors (D_2, 5-HT_{2A}, α_1) to which the reference compounds and our original series of arylpiperazines **7** with partial 5-HT_{1A} agonist properties were known to bind. The 1-(2-methoxyphenyl)piperazine derivative **9a** is a prototype of a compound from this original series. It is a rather weak ligand with equipotent affinity at both σ sites (Table 1), but it has high affinity for 5-HT_{1A} receptors, as previously reported.[18] Generally, **9a** possesses quite high affinity for all receptors measured (Table 3), especially noradrenergic α_1 adrenoceptors. Replacement of the piperazine ring with a piperidine ring (compound **9b**, Table 1) improved affinity to both σ sites. However, affinities for other receptors were only slightly weakened (Table 3). Removing the 2-methoxy substituent (compound **9d**) or replacing it with a 4-fluorine atom, as in the derivatives **9c**, **9e**, or **9f**, all resulted in potent σ ligands with almost equal affinity for both σ sites. The unsubstituted 4-phenylpiperidine **9d** was the most potent derivative with subnanomolar affinities. However, these compounds without substituents at the indole nitrogen atom also retained considerable affinity for the classical receptors, as indicated in Table 3. Compared to the reference compounds (Table 1), these derivatives were very potent and the σ_2 component was generally more predominant. All the reference compounds show preference for the σ_1 binding site except DTG, (−)-pent-

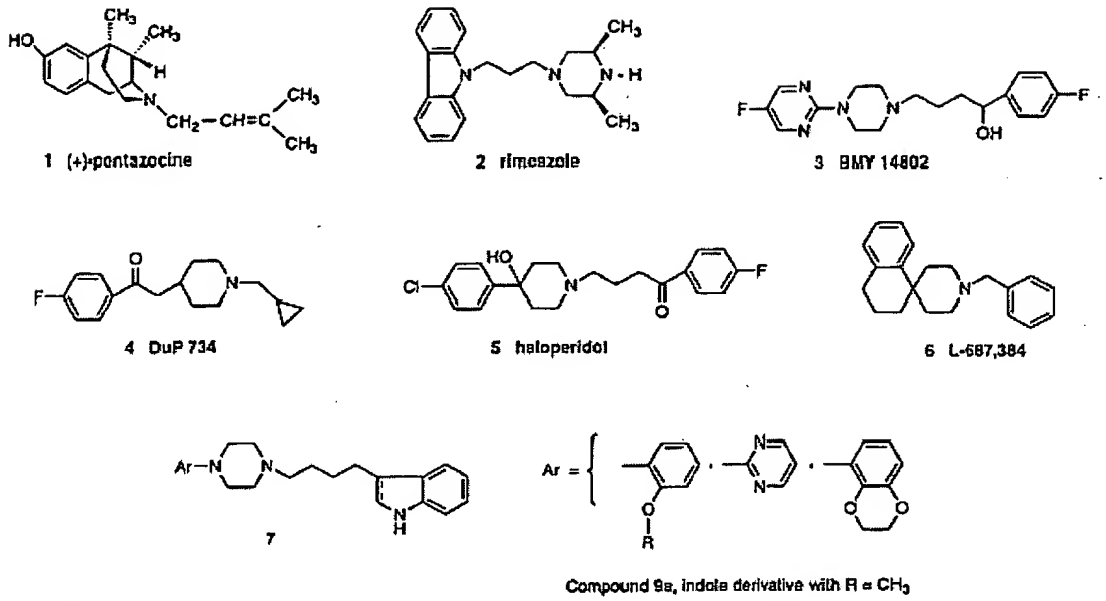


Figure 1. Reference and lead compounds.

Table 3. Selectivity of Selected σ Ligands and Reference Compounds

	binding affinities (IC_{50} values, nM)[a]			
compd	[^{3}H]-8-OHDPAT 5-HT_{1A}	[^{3}H]ketanserin 5-HT_{2A}	[^{3}H]spiroperidol D_2	[^{3}H]prazosine α_1
9a	17.	150.	15.	2.7
9b	56.	83.	20.	11.
9c	37.	14.	180.	12.
9d	110.	25.	45	14.
9e	27.	34.	160.	20.
9f	71.	27.	51.	24.
11a	22000.	270.	4200.	220.
11b	>10000	250.	3000.	420
12a	130.	360.	1300.	30.
14f	21000.	2000.	800.	330.
15a	>10000.	3700.	8000.	350.
3 BMY 14802	320.	830.	8400.	1500.
4 DuP 734	44000.	66.	640.	21.
5 haloperidol	3200.	55.	7.5	18.
6 L-687,384	>100000.	7100.	3700.	1000.

[a] Method for determination of IC_{50} values is described in footnote to Table 1. The following sd ratios were obtained: 5-HT_{1A} 1.4 (n = 100); D_2 1.7 (n = 38); α_1 1.4 (n = 44); 5-HT_{2A} 1.5 (n = 30).

azocine, and **2** that have equipotent but moderate to low affinities for both subtypes of σ sites (Table 1). The 4-phenacylpiperidine derivative **4** has additional binding to 5-HT_{2A} and α_1 receptors (Table 3). It has been reported that replacing the fluorine atom of **4** with a cyano group results in a more selective σ ligand.[15] The spirotetralin derivative from Merck, L-687,384 (**6**),[31] is a very potent and selective σ_1 ligand. Compound **3** is weak, and interaction with 5-HT_{1A} receptors (Table 3) cannot be ruled out as being responsible for its pharmacological properties.[32]

The next step was the introduction of a 4-fluorophenyl substituent at the indole nitrogen atom (compounds **11a–d**) that resulted in total elimination of the serotonin 5-HT_{1A} component (Table 3). Affinities for 5-HT_{2A}, D_2, and α_1 receptors were also considerably reduced, although less dramatically than 5-HT_{1A} receptor binding. Compounds **11a**, **11c**, and **11d** were very potent σ_2 ligands, while the piperazine derivative **11b** was less potent. Interestingly, all 1-(4-fluorophenyl)-substituted derivatives displayed pronounced preference for the σ_2 binding site with selectivity ratios of 40–100. The best ratios reported previously were below 10 and for compounds with much lower affinity for the receptor.[7]

All the spiropiperidines with a C-4 spacer chain had subnanomolar σ_2 binding affinities (Table 2), while shorter chain lengths (compounds **14g–i**) seem to reduce potency by a factor of about 10. Furthermore, all the spiropiperidines, except the C-1 derivative **14i**, show selectivity for σ_2 versus σ_1 binding sites. The 4-[1-(4-fluorophenyl)-1*H*-indol-3-yl]-1-butyl-substituted derivative **14f** was again the most selective σ_2 compound with a selectivity ratio of 140. As seen for the nonspiro derivatives, the 1-unsubstituted indole **12a** had considerable binding to the classical receptors (Table 3). These binding affinities were efficiently reduced by introduction of 1-substituents in the indole (compd **14f**, Table 3). Spirotetralin and spiroindan derivatives with high σ binding affinity have been synthesized by Chambers et al.[31] However, no indication of σ_1 or σ_2 preference were given for these series of compounds. [^{3}H]DTG labeling of guinea pig cerebellum was used for radioligand binding. Probably this assay measures both σ_1 and σ_2. As the compound 1′-benzyl-3,4-dihydrospiro-

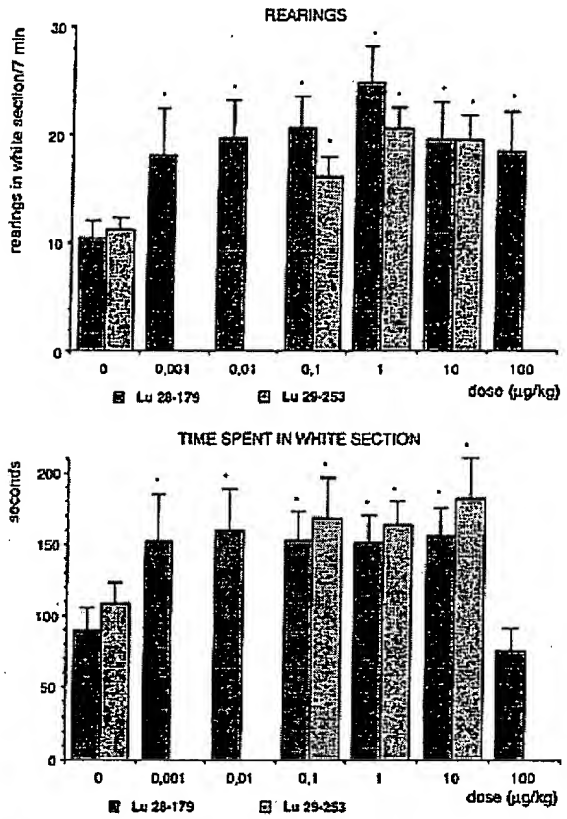


Figure 2. Effects of compounds **11a** and **14f** in the black/white exploration test in rats. Rearings in the white section is counted during a 7 min period, and the total time spent in the white section is measured. *p < 0.05 (one-way ANOVA, Dunnetts test) compared to control.

[naphthalene-1(2*H*),4′-piperidine] (**6**, L-687,384)[33] has been studied in some detail, we prepared and tested this spiro derivative. Surprisingly, **6** is a very potent and specific σ_1 ligand contrary to the indole derivatives within the present series. Given this result, one might speculate whether a benzylic type of substituents favor σ_1 affinity. Within our series we observed that the corresponding C-1 derivative **14i** was the most potent σ_1 ligand among homologous spiropiperidines (**14f–i**) and, contrary to all the other spiro derivatives, was equipotent for the two σ binding sites. The σ_1/σ_2 affinities and selectivities related to phenylalkyl chain lengths will be further discussed in part 2 of this series of articles.

Compounds **11a** (Lu 29-253) and **14f** (Lu 28-179) have been investigated further in various animal models predictive for anxiolytic activity. Exploratory behavior of rodents in a black and white, two-compartment test box is reduced in the brightly lit, white compartment and the animals spend more time exploring the dark compartment. Facilitation of the explorative behavior in the white compartment is suggested to reflect anxiolytic activity.[39] In the black/white box exploration test both compounds were active over a large dose range (Figure 2). The total time spent in the white compartment and the number of rearings in this compartment were both significantly increased.[34,35] The lowest effective dose tested for Lu 28-179 was 0.001 µg/kg, while Lu 29-253 was effective in doses at least from 0.1 µg/kg. Ex vivo ^{3}H-DTG binding in rats showed that Lu 28-179 has excellent CNS penetration with optimal effect about 3–6 h both after subcutaneous (3.0 µmol/kg) and peroral (3.0 µmol/kg) administration. A half-life of about 20 h in the CNS was also demonstrated by ex vivo binding measurements. The long half-life of this compound was also reflected in the black/white box exploration test, in which potent activity was still present 24 h after the administration.[36] Papers presenting the anxiolytic potential of Lu 28-179 in other test models are in preparation.[36] Compared to certain benzodiazepines, such as diazepam, the anxiolytic effect was more pronounced and no sedation was observed.

In conclusion, the present study has provided very potent σ ligands with a preference for the σ_2 binding site. IC_{50} values well below 1 nM were achieved for a majority of the compounds. Compound **15a** was the most selective σ_2 ligand, compared to its σ_1 affinity, with a ratio of about 500. However, compounds **11a** and **14f** were also very selective with selectivity ratios of 60 and 140, respectively. Compared to reference compounds these selectivities are quite outstanding. Selectivity with respect to a large number of receptors and transporter systems was also measured for these two compounds. No affinity of any significance (IC_{50} values > 100 nM) was found. Determination of intrinsic activity, i.e., the agonist/antagonist profile of the present series of compounds, still awaits proper biological methods. Whether the relaxation reported with (−)-pentazocine in humans is related to its σ_2 component can only be clarified by testing a more specific σ_2 ligand for anxiety in the clinic.

Experimental Section

Melting points were determined on a Büchi SMP-20 apparatus and are uncorrected. ^{1}H NMR spectra were recorded of all novel compounds at 250 MHz on a Bruker AC 250 spectrometer. Deuterated chloroform (99.8% D) or dimethyl sulfoxide (99.9% D) were used as solvents. TMS was used as internal reference standard. Chemical shift values are expressed in ppm values. The following abbreviations are used for multiplicity of NMR signals: s = singlet, d = doublet, t = triplet, q = quartet, qui = quintet, h = heptet, dd = double doublet, dt = double triplet, dq = double quartet, tt = triplet of triplets, m = multiplet. NMR signals corresponding to acidic protons are generally omitted. Content of water in crystalline compounds was determined by Karl Fischer titration. Microanalyses were performed by Lundbeck Analytical Department and results obtained were within ±0.4% of the theoretical values. Standard workup procedures refer to extraction with the indicated organic solvent from proper aqueous solutions, drying of combined organic extracts (anhydrous $MgSO_4$ or Na_2SO_4), filtering, and evaporation of the solvent in vacuo. For column chromatography, silica gel of type Kieselgel 60, 230–400 mesh ASTM, was used.

3-Indole-ω-alkanols (8). 4-(1*H*-Indol-3-yl)-1-butanol (**8a**) was prepared following a modification of a literature procedure.[24] Gaseous HCl was bubbled through a solution of 4-(1*H*-indol-3-yl)butanoic acid (100 g, 0.49 mol) in methanol (1 L) until saturation was achieved. The mixture was stirred at room temperature for 1.5 h. Methanol was evaporated in vacuo. The remaining oil was dissolved in diethyl ether (500 mL), washed with brine (2 × 100 mL), and dried (anhydrous $MgSO_4$). The solvent was evaporated leaving the indole-substituted butanoic acid methyl ester as a semisolid material: yield 103 g (96%). An analytical sample was recrystallized from *n*-heptane: mp 59–60 °C; ^{1}H NMR ($CDCl_3$) δ 2.10 (qui, 2H), 2.40 (t, 2H), 2.85 (t, 2H), 3.70 (s, 3H), 7.00 (d, 1H), 7.10–7.25 (m, 2H), 7.35 (d, 1H), 7.65 (d, 1H), 8.00 (broad s, 1H). A solution of the methyl ester (100 g, 0.46 mol) in dry tetrahydrofuran (THF) (1 L) was added dropwise to a suspension of $LiAlH_4$ (25 g, 0.66 mol) in dry THF (1 L) at such a rate that the temperature was maintained at about 40 °C. After

the mixture was stirred for an additional 40 min, water (25 mL) was added cautiously to the cooled solution (below 10 °C). Under vigorous stirring of the solution, concentrated aqueous NaOH solution (25 mL) was added dropwise. Inorganic salts were filtered off, and the solvents were evaporated in vacuo. The remaining oil was dissolved in dichloromethane (500 mL) and dried (anhydrous $MgSO_4$). The crude alcohol that was left after evaporation of dichloromethane was used without further purification: yield 88 g (100%); ^1H NMR ($CDCl_3$) δ 1.60–1.85 (m, 4H), 2.80 (t, 2H), 3.70 (t, 2H), 6.95 (d, 1H), 7.10–7.25 (m, 2H), 7.35 (d, 1H), 7.65 (d, 1H), 8.05 (broad s, 1H).

In a corresponding way **3-(1*H*-indol-3-yl)-1-propanol (8b)**[24] and **2-(1*H*-indol-3-yl)ethanol (8c)** were prepared.[25]

N-Phenylpiperazine, *N*-(4-fluorophenyl)piperazine, *N*-(2-methoxyphenyl)piperazine, and 4-phenylpiperidine were all commercially available. **4-(4-Fluorophenyl)piperidine** was prepared as follows. A mixture of 4-fluorobenzaldehyde (230 g, 1.85 mol) and ethyl acetoacetate (480 g, 3.69 mol) was cooled to 0 °C, and piperidine (25 mL) was added dropwise during 0.5 h. The mixture was left at room temperature for 3 days. The resulting solid product was dissolved in ethanol (1.0 L) and refluxed. After cooling to room temperature the crystalline product was filtered off and dried in vacuo: yield 463 g (69%); mp 160 °C. This product (450 g, 1.23 mol) was added portionwise to a solution of KOH (350 g) in water (300 mL) kept at 85–90 °C. The mixture was finally stirred for another 2 h at 80–85 °C. Ice (2 kg) and ethyl acetate (500 mL) were added. The aqueous phase was separated, and pH was adjusted to 1 by cautious addition of concentrated aqueous HCl (CO_2 evolves). The precipitated 3-(4-fluorophenyl)-glutaric acid was filtered off, washed with water, and dried: yield 207 g (75%); mp 141–143 °C. A mixture of the glutaric acid derivative (110 g, 0.49 mol) and urea (34 g, 0.57 mol) was heated at 160–180 °C for 2 h. After cooling below 80 °C ethanol (250 mL) was added and the mixture was refluxed for 10 min. The precipitated 4-(4-fluorophenyl)-2,6-piperidinedione was collected after cooling to 0 °C: yield 86 g (86%); mp 199 °C. To a suspension of $LiAlH_4$ (50 g, 1.32 mol) in dry THF (1 L) was added all of the piperidinedione (0.42 mol) in small portions at 40–60 °C. The resulting mixture was finally refluxed for 1.5 h. Water (20 mL), concentrated aqueous NaOH (20 mL), and water (200 mL) were sequentially added with caution. Inorganic salts were filtered off and the solvents evaporated in vacuo. The remaining oil was dissolved in dichloromethane (500 mL) and dried (anhydrous Na_2SO_4), and the solvent was evaporated, leaving 72 g (95%) of the 4-(4-fluorophenyl)piperidine; ^1H NMR ($CDCl_3$) δ 1.60 (dq, 2H), 1.80 (broad d, 2H), 2.35 (s, 1H), 2.60 (tt, 1H), 2.70 (dt, 2H), 3.20 (broad d, 2H), 7.00 (t, 2H), 7.10–7.20 (m, 2H).

In a similar way **4-(2-methoxyphenyl)piperidine** was prepared: mp 204–210 °C (diisopropyl ether); ^1H NMR ($CDCl_3$) δ 1.65 (dq, 2H), 1.85 (broad d, 2H), 2.80 (tt, 1H), 3.10–3.30 (m, 4H), 3.85 (s, 3H), 6.90 (d, 1H), 7.00 (t, 1H), 7.15–7.25 (m, 2H). **Spiro[isobenzofuran-1(3*H*),4'-piperidine]** was prepared as described by Marxer et al.[26] The synthesis of **4-(4-fluorophenyl)-1,2,3,6-tetrahydropyridine** was a modification of a literature method[27] as follows. To a solution of 1.6 M *n*-butyllithium in *n*-hexane (250 mL) in dry diethyl ether (300 mL) kept at −50 to −40 °C was added dropwise a solution of 4-bromofluorobenzene (73 g, 0.42 mol) in dry diethyl ether (150 mL). After the mixture was stirred for another 40 min at −50 °C, a solution of 1-benzyl-4-piperidone (57 g, 0.30 mol) in dry diethyl ether (200 mL) was added dropwise. The mixture was further stirred until the temperature reached −10 °C. Diluted hydrochloric acid was added. The organic phase was discarded. To the partly precipitated hydrochloric salt remaining in the aqueous phase was added diethyl ether followed by aqueous NH_4OH until pH > 9. The organic phase was separated and worked up leaving 1-benzyl-4-(4-fluorophenyl)-4-piperidinol as an oil: yield 82 g (97%) ^1H NMR ($CDCl_3$) δ 1.70 (dq, 2H), 1.80 (broad s, 1H), 2.15 (dt, 2H), 2.50 (dt, 2H), 2.80 (broad d, 2H), 3.60 (s, 2H), 7.05 (t, 2H), 7.25–7.40 (m, 5H), 7.50 (dd, 2H). The 4-piperidinol (82 g, 0.29 mol) was refluxed in trifluoroacetic acid (500 mL) for 1.5 h. Most of the trifluoroacetic acid was evaporated in vacuo. To the remaining oil was added diethyl ether, water, and aqueous NH_4OH until pH > 9. The organic phase was worked up as above: yield 76 g (96%) of 1-benzyl-4-(4-fluorophenyl)-1,2,3,6-tetrahydropyridine; ^1H NMR ($CDCl_3$) δ 2.50–2.60 (m, 2H), 2.75 (t, 2H), 3.15 (q, 2H), 3.65 (s, 2H), 6.00 (h, 1H), 7.00 (t, 2H), 7.30–7.50 (m, 7H), 7.50 (dd, 2H). To a solution of all the thus isolated oil (0.28 mol) in 1,1,1-trichloroethane (760 mL) kept at reflux was added dropwise 2,2,2-trichloroethyl chloroformate (45 mL) during 40 min. The mixture was refluxed for 3 h. The solvent was removed by evaporation, and the carbamate was purified by filtering through silica gel using dichloromethane/heptane 1:3 as eluent: yield 90 g (93%); ^1H NMR ($CDCl_3$) δ 2.50–2.60 (m, 2H), 3.70–3.80 (m, 2H), 4.20 (broad d, 2H), 4.80 (s, 2H), 6.00 (broad s, 1H), 7.05 (t, 2H), 7.45 (dd, 2H). The carbamate group was removed by addition of Zn-powder (100 g, 1.53 mol) to a solution of the carbamate derivative (50 g, 0.14 mol) in a mixture of acetic acid (450 mL) and water (50 mL) kept at 45 °C. Small lots of Zn were added during 1.5 h. Inorganic salts were filtered off, and the solvents were evaporated in vacuo. The 4-(4-fluorophenyl)-1,2,3,6-tetrahydropyridine was isolated as an oil by extraction from an alkaline aqueous phase with ethyl acetate, according to the general workup procedure: yield 23 g (93%); ^1H NMR ($CDCl_3$) δ 1.75 (s, 1H), 2.35–2.45 (m, 2H), 3.10 (t, 2H), 3.50 (q, 2H), 6.00–6.10 (m, 1H), 6.95 (t, 2H), 7.30–7.40 (m, 2H).

3-(4-Fluorophenyl)-8-azabicyclo[3.2.1]-oct-2-ene was prepared analogously: To a solution of 1.6 M *n*-butyllithium in *n*-hexane (500 mL) in dry diethyl ether (600 mL) kept at −45 °C was added dropwise a solution of 4-bromofluorobenzene (145 g, 0.84 mol) in dry diethyl ether (350 mL). After stirring for another 20 min at −50 °C, a solution of 8-methyl-8-azabicyclo[3.2.1]octan-3-one (85 g, 0.64 mol) in dry diethyl ether (200 mL) was added dropwise. The mixture was further stirred until the temperature reached −20 °C. Diluted hydrochloric acid was added. The organic phase was discarded. To the partly precipitated hydrochloric salt remaining in the aqueous phase was added diethyl ether followed by aqueous NH_4OH until pH > 9. The organic phase was separated and worked up leaving 3-(4-fluorophenyl)-8-methyl-8-azabicyclo-[3.2.1]octan-3-ol that crystallized from diethyl ether: yield 96 g (64%); mp 169 °C. All of the 4-piperidinol (96 g, 0.41 mol) was refluxed in trifluoroacetic acid (500 mL) for 1 h. Most of the trifluoroacetic acid was evaporated in vacuo. To the remaining oil was added diethyl ether, water, and aqueous NH_4OH until pH > 9. The organic phase was worked up as above: yield 87 g (98%) of 3-(4-fluorophenyl)-8-methyl-8-azabicyclo[3.2.1]oct-2-ene; mp 62–63 °C (from 2-propyl ether/*n*-heptane, 1:1). To a solution of the thus isolated azabicyclo-[3.2.1]oct-2-ene (54 g, 0.25 mol) in 1,1,1-trichloroethane (550 mL) kept at 70 °C was added dropwise 2,2,2-trichloroethyl chloroformate (38 mL) during 2 h. The mixture was refluxed for 3 h. The solvent was removed by evaporation, and the carbamate was purified by filtering through silica gel using dichloromethane as eluent: yield 59 g (64%); ^1H NMR ($CDCl_3$) δ 1.70–1.85 (m, 1H), 1.95–2.40 (m, 4H), 3.10 (broad d, 1H), 4.55–4.80 (m, 4H), 6.35 (s, 1H), 7.00 (t, 2H), 7.35 (dd, 2H). The carbamate group was removed by addition of Zn powder (40 g, 0.61 mol) to a solution of the carbamate derivative (17 g, 0.045 mol) in a mixture of acetic acid (170 mL) and water (20 mL) kept at 45 °C. Small lots of Zn were added during 1.5 h. Inorganic salts were filtered off, and the solvents were evaporated in vacuo. The 3-(4-fluorophenyl)-8-azabicyclo-[3.2.1]oct-2-ene was isolated as an oil by extraction from an alkaline aqueous phase with ethyl acetate according to the general workup procedure: yield 7 g as an oil (76%); ^1H NMR ($CDCl_3$) δ 1.60–1.70 (m, 1H), 1.80–2.20 (m, 5H), 2.45 (d, 1H), 2.85 (dd, 1H), 3.80–3.90 (m, 2H), 6.45 (d, 1H), 6.95 (t, 2H), 7.25–7.35 (m, 2H). The oxalate salt crystallized from acetone: mp 154–155 °C.

General Procedure for the Synthesis of 1-Unsubstituted 3-[4-(4-Phenyl-1-piperidinyl)-1-butyl]-1*H*-indoles and Corresponding 1,2,3,6-Tetrahydropyridiny, and Piperazinyl Derivatives, 9 (Table 1). 3-[4-[4-(2-Methoxyphenyl)-1-piperazinyl]-1-butyl]-1*H*-indole (9a). A solution of 4-(1*H*-indol-3-yl)-1-butanol, 8a (316 g, 1.67 mol), and triethylamine (340 mL) in dichloromethane (3.2 L) was cooled to 0 °C, and methanesulfonyl chloride (170 mL, 2.20 mol) dissolved in dichloromethane (300 mL) was added dropwise while keeping the temperature below 5 °C. After the mixture

was stirred for an additional 45 min at 15 °C, water (2.0 L) was added. The organic phase was separated and worked up according to the standard procedure leaving the methanesulfonate ester that was used without further purification. For storage the ester was kept in a refrigerator. Yield 450 g (100%) as an oil. A mixture of the methanesulfonate ester (8 g, 0.030 mol), *N*-(2-methoxyphenyl)piperazine (5.5 g, 0.029 mol), and potassium carbonate (6.0 g, 0.043 mol) in acetone (100 mL) was refluxed for 24 h. The acetone was evaporated, and the residue was dissolved in water (200 mL) and diethyl ether (200 mL). The aqueous phase was made acidic by addition of acetic acid, and the organic phase was separated and discarded. Aqueous NH_4OH was added to adjust the pH to > 10. Extraction with diethyl ether and workup as above afforded 10 g of crude **9a**. Pure title compound crystallized from diethyl ether: yield 7.8 g (74%); mp 113–115 °C; ^1H NMR ($CDCl_3$) δ 1.60–1.85 (m, 4H), 2.40 (t, 2H), 2.65 (broad s, 4H), 2.85 (t, 2H), 3.10 (broad s, 4H), 3.85 (s, 3H), 6.90 (t, 1H), 6.90–7.05 (m, 4H), 7.10–7.20 (m, 2H), 7.35 (d, 1H), 7.60 (d, 1H), 8.00 (broad s, 1H). Anal. ($C_{23}H_{28}N_3O$) C, H, N.

In a corresponding way the following indoles **9** were prepared.

3-[4-[4-(2-Methoxyphenyl)-1-piperidinyl]-1-butyl]-1*H*-indole hemioxalate (9b): mp 183–188 °C (acetone); ^1H NMR (DMSO-d_6) δ 1.60–1.80 (m, 8H), 2.50–2.60 (m, 2H), 2.65–2.75 (m, 4H), 2.95–3.05 (m, 1H), 3.25 (d, 2H), 3.75 (s, 3H), 6.85–7.20 (m, 7H), 7.35 (d, 1H), 7.50 (d, 1H), 10.80 (s, 1H). Anal. ($C_{24}H_{30}N_2O$·hemioxalate) C, H, N.

3-[4-[4-(4-Fluorophenyl)-1-piperazinyl]-1-butyl]-1*H*-indole (9c): mp 124–126 °C (diisopropyl ether); ^1H NMR ($CDCl_3$) δ 1.55–1.85 (m, 4H), 2.45 (t, 2H), 2.60 (t, 4H), 2.80 (t, 2H), 3.15 (t, 4H), 6.80–7.00 (m, 4H), 6.95 (s, 1H), 7.05–7.20 (m, 2H), 7.35 (d, 1H), 7.60 (d, 1H), 8.00 (broad s, 1H). Anal. ($C_{22}H_{26}FN_3$) C, H, N.

3-[4-(4-Phenyl-1-piperidinyl)-1-butyl]-1*H*-indole (9d): mp 131–132 °C (diisopropyl ether); ^1H NMR ($CDCl_3$) δ 1.60–1.85 (m, 8H), 2.05 (dt, 2H), 2.40–2.55 (m, 3H), 2.80 (t, 2H), 3.05 (broad d, 2H), 6.95 (s, 1H), 7.05–7.40 (m, 8H), 7.60 (d, 1H), 7.95 (broad s, 1H). Anal. ($C_{23}H_{28}N_2$) C, H, N.

3-[4-[4-(4-Fluorophenyl)-1-piperidinyl]-1-butyl]-1*H*-indole (9e): mp 92–93 °C (washed with *n*-heptane); ^1H NMR ($CDCl_3$) δ 1.60–1.90 (m, 8H), 2.05 (dt, 2H), 2.40–2.55 (m, 3H), 2.80 (t, 2H), 3.10 (broad d, 2H), 6.95–7.05 (m, 3H), 7.10–7.25 (m, 4H), 7.35 (d, 1H), 7.65 (d, 1H), 8.05 (broad s, 1 H). Anal. ($C_{23}H_{27}FN_2$) C, H, N.

3-[4-[4-(4-Fluorophenyl)-1-(1,2,3,6-tetrahydro)pyridinyl]-1-butyl]-1*H*-indole (9f): mp 124–125 °C (washed with diethyl ether); ^1H NMR ($CDCl_3$) δ 1.60–1.80 (m, 4H), 2.45–2.55 (m, 4H), 2.70 (t, 2H), 2.80 (t, 2H), 3.15 (q, 2 H), 6.00 (broad s, 1H), 6.95–7.05 (m, 3H), 7.05–7.20 (m, 2H), 7.30–7.40 (m, 3H), 7.65 (d, 1H), 8.00 (broad s, 1H). Anal. ($C_{23}H_{25}FN_2$) C, H, N.

General Procedure for the Synthesis of 1-(4-Fluorophenyl)-Substituted 3-[4-(4-Phenyl-1-piperidinyl)-1-butyl]-1*H*-indoles and Corresponding Piperazinyl Derivatives, 11 (Table 1). 4-[1-(4-Fluorophenyl)-1*H*-indol-3-yl]-1-butanol (10a). A mixture of 4-(1*H*-indol-3-yl)-1-butanol, **8a** (120 g, 0.63 mol), potassium carbonate (110 g, 0.80 mol), 4-fluoroiodobenzene (240 g, 1.09 mol), CuI (30 g), and ZnO (7.5 g) in 1-methyl-2-pyrrolidinone (NMP) (1.2 L) was heated at 155 °C for 6 h. After cooling, precipitated inorganic salts were filtered off. Diethyl ether (500 mL) and diluted aqueous NH_4OH (4.0 L) were added. The organic phase was separated, washed with saturated brine, and subsequently worked up as above. The crude butanol derivative was purified by column chromatography on silica gel (eluted with diethyl ether): yield of pure title compound **10a** as an oil 104 g (58%); ^1H NMR ($CDCl_3$) δ 1.65–1.95 (m, 5H), 2.90 (t, 2H), 3.75 (t, 2H), 7.10 (s, 1H), 7.20 (t, 2H), 7.25 (d, 1H), 7.40–7.50 (m, 3H), 7.70 (d, 1H).

1-(4-Fluorophenyl)-3-[4-[4-(4-fluorophenyl)-1-piperidinyl]-1-butyl]-1*H*-indole Hemifumarate (11a). A solution of 4-[1-(4-fluorophenyl)-1*H*-indol-3-yl]-1-butanol, **10a** (104 g, 0.37 mol), and triethylamine (75 mL) in dichloromethane (1.0 L) was cooled to 0 °C and methanesulfonyl chloride (30 mL, 0.39 mol) dissolved in dichloromethane (150 mL) was added dropwise while keeping the temperature below 10 °C. After the mixture was stirred for additional 1.5 h at room temperature, water (1.5 L) was added. The organic phase was finally worked up, according to the standard procedure, leaving 128 g (95%) of the methanesulfonate ester that was used without further purification. To the methanesulfonate ester (128 g, 0.35 mol) in methyl isobutyl ketone (MIBK) (1 L) was added 4-(4-fluorophenyl)piperidine (as the trifluoroacetic acid salt) (87 g, 0.30 mol) and potassium carbonate (90 g, 0.65 mol). The mixture was heated at reflux temperature for 16 h. After cooling, inorganic salts were filtered off and MIBK evaporated in vacuo. Ethyl acetate (500 mL) and water (2.0 L) were added, and the organic phase was separated and worked up as above. The remaining crude product was dissolved in boiling ethanol (400 mL). After the mixture cooled in an ice bath, the precipitated product was filtered off: yield 78 g (58%). The hemifumarate salt **11a** was crystallized from ethanol: mp 157 °C; ^1H NMR (DMSO-d_6) δ 1.55–1.80 (m, 8H), 2.25 (dt, 2H), 2.60 (broad t, 2H), 2.75 (t, 2H), 3.10 (broad d, 2H), 6.55 (s, 1H), 7.05–7.25 (m, 6H), 7.35 (d, 1H), 7.40 (s, 1H), 7.45 (d, 1H), 7.55–7.65 (m, 3H). Anal. ($C_{29}H_{30}F_2N_2$·hemifumarate) C, H, N.

In a corresponding way the following 1-(4-fluorophenyl)-substituted indoles **11** were prepared.

1-(4-Fluorophenyl)-3-[4-[4-(4-fluorophenyl)-1-piperazinyl]-1-butyl]-1*H*-indole (11b): mp 65–66 °C (ethyl acetate); ^1H NMR ($CDCl_3$) δ 1.60–1.90 (m, 4H), 2.50 (t, 2H), 2.60 (t, 4H), 2.85 (t, 2H), 3.10 (t, 4H), 6.85–7.00 (m, 4H), 7.10 (s, 1H), 7.15–7.25 (m, 4H), 7.40–7.50 (m, 3H), 7.65 (d, 1H). Anal. ($C_{28}H_{29}F_2N_3$) C, H, N.

1-(4-Fluorophenyl)-3-[4-(4-phenyl-1-piperidinyl)-1-butyl]-1*H*-indole fumarate (11c): mp 171–173 °C (ethanol); ^1H NMR (DMSO-d_6) δ 1.60–1.85 (m, 8H), 2.35–2.50 (m, 2H), 2.55–2.85 (m, 5H), 3.20 (broad d, 2H), 6.55 (s, 2H), 7.05–7.50 (m, 11H), 7.55–7.70 (m, 3H). Anal. ($C_{29}H_{31}FN$·fumarate) C, H, N.

1-(4-Fluorophenyl)-3-[4-(4-phenyl-1-piperazinyl)-1-butyl]-1*H*-indole difumarate (11d): mp 120–122 °C (ethanol); ^1H NMR (DMSO-d_6) δ 1.50–1.70 (m, 4H), 2.50 (t, 2H), 2.65 (broad t, 4H), 2.80 (t, 2H), 3.15 (broad t, 4H), 6.60 (s, 4H), 6.80 (t, 1H), 6.90 (d, 2H), 7.10–7.25 (m, 4H), 7.40–7.50 (m, 4H), 7.55–7.65 (m, 3H). Anal. ($C_{28}H_{30}FN_3$·difumarate) C, H, N.

1′-[4-(1*H*-Indol-3-yl)-1-butyl]spiro[isobenzofuran-1(3*H*),4′-piperidine] (12a). A suspension of the methanesulfonate ester (20 g, 0.075 mol) of 4-(1*H*-indol-3-yl)-1-butanol, **8a**, spiro[isobenzofuran-1(3*H*),4′-piperidine] (15 g, 0.079 mol), and potassium carbonate (11 g, 0.080 mol) in MIBK (400 mL) was refluxed overnight. After the mixture was cooled to room temperature, inorganic salts were filtered off. The remaining oil was purified by filtering through silica gel (eluted with 4% triethylamine in a 3:2 mixture of ethyl acetate and *n*-heptane): yield 21 g (77%) of crude title compound that was stirred with diethyl ether and filtered off; mp 150–155 °C; ^1H NMR ($CDCl_3$) δ 1.60–1.85 (m, 6H), 2.00 (dt, 2H), 2.40 (dt, 2H), 2.45–2.55 (m, 2H), 2.85 (t, 2H), 2.65–2.95 (m, 2H), 5.05 (s, 2H), 6.95 (d, 1H), 7.00–7.25 (m, 6H), 7.30 (d, 1H), 7.55 (d, 1H), 8.05 (broad s, 1H). Anal. ($C_{24}H_{28}N_2O$) C, H, N.

Procedures for the Syntheses of 1-Substituted 3-(Spiro[isobenzofuran-1(3*H*),4′-piperidine]-1′-yl-1-butyl)-1*H*-indoles, 14 (Table 2). 1′-[4-(1-Methyl-1*H*-indol-3-yl)-1-butyl]-spiro[isobenzofuran-1(3*H*),4′-piperidine] Oxalate (14a). The sequence of the synthetic steps indicated in Scheme 2 was slightly changed in the preparation of compound **14a**. To a solution of 4-(1*H*-indol-3-yl)butanoic acid (24 g, 0.12 mol) in dry *N*,*N*-dimethylformamide (DMF) (200 mL) was slowly added potassium *tert*-butoxide (28 g, 0.26 mol). The mixture was cooled below 10 °C, and methyl iodide (60 mL, 0.96 mol) was added dropwise during 30 min. The mixture was finally stirred overnight at room temperature. It was poured into water (1 L) and diethyl ether (250 mL), and the organic phase was worked up as previously. The crude product was filtered through silica gel using dichloromethane as eluent, affording 18 g (65%) of pure methyl 4-(1-methyl-1*H*-indol-3-yl)butanoic acid ester as an oil: ^1H NMR ($CDCl_3$) δ 2.05 (p, 2H), 2.40 (t, 2H), 2.80 (t, 2H), 3.65 (s, 3H), 3.75 (s, 3H), 6.80 (s, 1H), 7.10 (t, 1H), 7.15–7.30 (m, 2H), 7.55 (d, 1H). A solution of the methyl ester (17 g, 0.073 mol) in dry THF (100 mL) was added dropwise to a suspension of $LiAlH_4$ (4.5 g, 0.12 mol) in dry THF (150 mL) at such a rate that the temperature was

maintained at about 40 °C. After the mixture was stirred for additional 1 h, water (5 mL) was added cautiously to the cooled solution (below 10 °C). Under vigorously stirring of the solution, concentrated aqueous NaOH solution (5 mL) was added dropwise. Inorganic salts were filtered off, and the solvents were evaporated in vacuo. The remaining oil was dissolved in dichloromethane (500 mL) and dried (anhydrous $MgSO_4$). The crude alcohol that was left after evaporation of dichloromethane was used without further purification: yield 14.9 g (100%); ^{1}H NMR ($CDCl_3$) δ 1.60–1.85 (m, 4H), 2.75 (t, 2H), 3.65 (t, 2H), 3.70 (s, 3H), 6.80 (s, 1H), 7.05 (t, 1H), 7.15–7.30 (m, 2H), 7.60 (d, 1H). The butanol was converted to the methanesulfonate ester, according to the procedure described above for the preparation of compound **9a**: yield 16 g (78%); ^{1}H NMR ($CDCl_3$) δ 1.75–1.90 (m, 4H), 2.80 (t, 2H), 2.90 (s, 3H), 3.70 (s, 3H), 4.20 (t, 2H), 6.80 (s, 1H), 7.10 (t, 1H), 7.15–7.30 (m, 2H), 7.55 (d, 1H). A mixture of the methanesulfonate ester (1.9 g, 0.0068 mol), spiro[isobenzofuran-1(3*H*),4′-piperidine] (1.5 g, 0.0079 mol), and potassium carbonate (1.5 g, 0.011 mol) was heated at reflux in MIBK (50 mL) for 16 h. After cooling to room temperature, inorganic salts were filtered off, MIBK was evaporated in vacuo, and the remaining crude title compound was purified by column chromatography on silica gel (eluted with 4% triethylamine in ethyl acetate and heptane, 2:3). The oxalate salt was finally crystallized from acetone: yield of **14a** 2.3 g (73%); mp 101–102 °C; ^{1}H NMR (DMSO-d_6) δ 1.60–1.80 (m, 6H), 2.25 (dt, 2H), 2.75 (t, 2H), 3.10 (broad t, 4H), 3.40 (broad d, 2H), 3.75 (s, 3H), 5.00 (s, 2H), 7.00 (t, 1H), 7.15 (s, 1H), 7.15 (d, 1H), 7.20–7.30 (m, 1H), 7.30–7.40 (m, 4H), 7.55 (d, 1H). Anal. ($C_{25}H_{30}N_2O$-oxalate) C, H, N.

1′-[4-(1-Acetyl-1*H*-indol-3-yl)-1-butyl]spiro[isobenzofuran-1(3*H*),4′-piperidine] Oxalate (14b). To a solution of 1′-[4-(1*H*-indol-3-yl)-1-butyl]spiro[isobenzofuran-1(3*H*),4′-piperidine], **12a** (1.8 g, 0.0050 mol), in dichloromethane (40 mL) were added tetrabutylammonium hydrogen sulphate (200 mg) and powdered sodium hydroxide (1 g). A solution of acetyl chloride (0.8 mL, 0.011 mol) in dichloromethane (10 mL) was added dropwise during 10 min below 25 °C. After the mixture was stirred for 1 h at room temperature, water (200 mL) was added. The organic phase was separated and worked up as previously. The crude title compound was subjected to column chromatography on silica gel (eluted with 4% triethylamine in ethyl acetate and heptane 2:3). The oxalate salt crystallized from acetone: yield of **14b** 0.9 g (37%); mp 139–140 °C; ^{1}H NMR (DMSO-d_6) δ 1.65–1.90 (m, 6H), 2.35 (dt, 2H), 2.65 (s, 3H), 3.05–3.20 (m, 4H), 3.45 (broad d, 2H), 5.05 (s, 2H), 7.20–7.30 (m, 6H), 7.60–7.70 (m, 2H), 8.30 (d, 1H). Anal. ($C_{25}H_{30}N_2O_5$-oxalate) C, H, N.

1′-[4-[1-Methylsulfonyl)-1*H*-indol-3-yl]-1-butyl]spiro[isobenzofuran-1(3*H*),4′-piperidine] Oxalate (14c). To a well-stirred suspension of a solution of NaOH (20 g) in water (20 mL), a solution of 4-(1*H*-indol-3-yl)-1-butanol, **8a** (4 g, 0.021 mol), in dichloromethane (60 mL), and tetrabutylammonium hydrogen sulphate (0.8 g) kept at 15 °C was added dropwise a solution of methanesulfonyl chloride (3.0 mL, 0.039 mol) in dichloromethane (25 mL) during 20 min. The temperature was gradually allowed to reach room temperature. Water (100 mL) was added, and the organic phase was separated and worked up as above. Pure methanesulfonate ester of 4-[1-methylsulfonyl]-1*H*-indol-3-yl]-1-butanol was obtained by column chromatography on silica gel (eluted with a 1:1:1 mixture of diethyl ether, dichloromethane, and heptane): yield 1.8 g (27%) as an oil; ^{1}H NMR ($CDCl_3$) δ 1.75–1.90 (m, 4H), 2.80 (t, 2H), 2.95 (s, 3H), 3.10 (s, 3H), 4.25 (t, 2H), 7.20 (s, 1H), 7.20–7.40 (m, 4H), 7.50 (d, 1H), 7.90 (d, 1H). A mixture of the methanesulfonate (1.8 g, 0.0052 mol), spiro[isobenzofuran-1(3*H*),4′-piperidine] (1.3 g, 0.0068 mol), and potassium carbonate (1 g, 0.0072 mol) in MIBK (50 mL) was heated at reflux for 16 h. After cooling, inorganic salts were filtered off, MIBK was evaporated in vacuo, and the remaining oil was subsequently subjected to column chromatography on silica gel (eluted with 4% triethylamine in ethyl acetate and heptane, 2:3). The oxalate salt crystallized from acetone: yield of **14c** 1.2 g (44%); mp 83–85 °C; ^{1}H NMR (DMSO-d_6) δ 1.65–1.85 (m, 6H), 2.35 (dt, 2H), 2.75 (t, 2H), 3.05–3.20 (m, 4H), 3.35 (s, 3H), 3.45 (broad d, 2H), 5.05 (s, 2H), 7.15–7.30 (m, 6H), 7.35 (d, 1H), 7.40 (s, 1H), 7.65 (d, 1H), 7.80 (d, 1H). Anal. ($C_{25}H_{30}N_2O_5S$-oxalate) C, H, N.

1′-[4-[1-(4-Tolylsulfonyl)-1*H*-indol-3-yl]-1-butyl]spiro[isobenzofuran-1(3*H*),4′-piperidine] Fumarate (14d). To a well-stirred mixture of a solution of NaOH (20 g) in water (20 mL), a solution of 1′-[4-(1*H*-indol-3-yl)-1-butyl]spiro[isobenzofuran-1(3*H*),4′-piperidine], **12a** (4.3 g, 0.012 mol) in dichloromethane (60 mL), and tetrabutylammonium hydrogen sulphate (0.8 g), kept at 15 °C, was added dropwise a solution of 4-toluenesulfonyl chloride (3.4 g, 0.018 mol) in dichloromethane (25 mL) during 20 min. The temperature was gradually allowed to reach room temperature. Water (100 mL) was added, and the organic phase was separated and worked up as above. Pure title compound was obtained by column chromatography on silica gel (eluted with 4% triethylamine in ethyl acetate and heptane 2:3). The fumarate salt crystallized from ethanol: yield of **14d** 2.2 g (29%); mp 202–204 °C; ^{1}H NMR (DMSO-d_6) δ 1.55–1.75 (m, 6H), 2.10 (dt, 2H), 2.50 (s, 3H), 2.50–2.75 (m, 6H), 3.00 (broad d, 2H), 5.00 (s, 2H), 6.60 (s, 2H), 7.20–7.40 (m, 8H), 7.60 (d, 2H), 7.80 (d, 2H), 7.90 (d, 1H). Anal. ($C_{31}H_{34}N_2O_5S$-fumarate) C, H, N.

1′-[4-(1-Benzyl-1*H*-indol-3-yl)-1-butyl]spiro[isobenzofuran-1(3*H*),4′-piperidine] Oxalate (14e). A solution of 4-(1*H*-indol-3-yl)-1-butanol, **8a** (5 g, 0.027 mol) in dry DMF (50 mL) was cooled to 10 °C. Potassium *tert*-butoxide (6.1 g, 0.054 mol) was added in small portions during 5 min. A solution of benzyl bromide (9.2 g, 0.054 mol) in dry DMF (10 mL) was added dropwise during 15 min. The temperature was raised to room temperature, and the mixture was stirred for an additional hour. Ethyl acetate (100 mL) and water (300 mL) were added. The organic phase was worked up as previously: yield of crude 4-(1-benzyl-1*H*-indol-3-yl)-1-butanol 10.9 g as an quite impure oil. It was not possible completely to distinguish the NMR signals of the butanol derivative from this mixture. All of the impure butanol derivative was converted into the *O*-methanesulfonate ester using the same procedure as described for the synthesis of compound **11a**: yield 5.7 g (59%) as an oil; ^{1}H NMR ($CDCl_3$) δ 1.65–1.90 (m, 4H), 2.80 (t, 2H), 2.90 (s, 3H), 4.20 (t, 2H), 5.20 (s, 2H), 6.85 (s, 1H), 7.05–7.40 (m, 8H) 7.60 (d, 1H). A mixture of the methanesulfonate ester (5.7 g, 0.016 mol), spiro[isobenzofuran-1(3*H*),4′-piperidine] (2.2 g, 0.012 mol), potassium carbonate (1.7 g, 0.012 mol), and a KI crystal was heated at reflux temperature in MIBK (75 mL) for 16 h. After cooling, inorganic salts were filtered off, and MIBK was evaporated in vacuo. The remaining crude product was purified by column chromatography on silica gel (eluted with 4% triethylamine in ethyl acetate and heptane, 1:1): yield 3.4 g (63%) as an oil. The oxalate salt crystallized from acetone: mp 167 °C; ^{1}H NMR (DMSO-d_6) δ 1.60–1.85 (m, 6H), 2.20 (dt, 2H), 2.70 (t, 2H), 3.00–3.20 (m, 4H), 3.40 (broad d, 2H), 5.00 (s, 2H), 5.35 (s, 2H), 7.00–7.10 (m, 2H), 7.10–7.35 (m, 11H), 7.40 (d, 1H), 7.55 (d, 1H). Anal. ($C_{31}H_{34}N_2O$-oxalate) C, H, N.

1′-[4-[1-(4-Fluorophenyl)-1*H*-indol-3-yl]-1-butyl]spiro[isobenzofuran-1(3*H*),4′-piperidine] Fumarate (14f). A mixture of the methanesulfonate ester (80 g, 0.22 mol) of 4-[1-(4-fluorophenyl)-1*H*-indol-3-yl]-1-butanol, **10a**, prepared as above in the synthesis of compound **11a**, spiro[isobenzofuran-1(3*H*),4′-piperidine] (50 g, 0.26 mol), and potassium carbonate (50 g, 0.36 mol) was heated at reflux temperature in MIBK (900 mL) for 16 h. After cooling, inorganic salts were filtered off, and MIBK was evaporated in vacuo. The crude title compound was purified by column chromatography on silica gel (eluted with 4% triethylamine in ethyl acetate and heptane, 2:3). The fumarate salt crystallized from ethanol: yield of **14f** 97 g (77%); mp 193 °C; ^{1}H NMR (DMSO-d_6) δ 1.65–1.85 (m, 6H), 2.15 (dt, 2H), 2.75 (dt, 2H), 2.80 (broad s, 4H), 3.15 (broad d, 2H), 4.95 (s, 2H), 6.55 (s, 2H), 7.10–7.60 (m, 13H). Anal. ($C_{30}H_{31}FN_2O$-fumarate) C, H, N.

In a corresponding way the following 1-(4-fluorophenyl)-substituted indoles **13** were prepared:

1′-[3-[1-(4-Fluorophenyl)-1*H*-indol-3-yl]-1-propyl]spiro[isobenzofuran-1(3*H*),4′-piperidine] oxalate (14g): mp 192–193 °C (acetone); ^{1}H NMR (DMSO-d_6) δ 1.80 (broad d, 2H), 2.00–2.35 (m, 4H), 2.80 (t, 2H), 3.05–3.30 (m, 4H), 3.50 (broad d, 2H), 5.05 (s, 2H), 7.10–7.60 (m, 13H). Anal. ($C_{29}H_{27}FN_2O$-oxalate) C, H, N.

1'-[2-[1-(4-Fluorophenyl)-1*H*-indol-3-yl]-1-ethyl]spiro[isobenzofuran-1(3*H*),4'-piperidine] oxalate (14h): mp 204–205 °C (acetone); ^{1}H NMR (DMSO-d_6) δ 1.85 (broad d, 2H), 2.35 (dt, 2H), 3.10–3.35 (m, 4H), 3.40 (t, 2H) 3.50 (broad d, 2H), 5.05 (s, 2H), 7.10–7.60 (m, 11H), 7.75 (d, 1H). Anal. ($C_{28}H_{27}FN_2O$·oxalate) C, H, N.

1'-[[1-(4-Fluorophenyl)-1*H*-indol-3-yl]methyl]spiro[isobenzofuran-1(3*H*),4'-piperidine] 1.25Fumarate (14i). A mixture of 1*H*-indole-3-carboxaldehyde (9 g, 0.062 mol), 4-fluoroiodobenzene (27 g, 0.12 mol), potassium carbonate (10 g, 0.072 mol), CuI (4 g), and ZnO (2 g) was heated in NMP (100 mL) at 160 °C for 20 h. After cooling, diluted aqueous NH_4OH (500 mL) and diethyl ether (200 mL) were added. The organic phase was separated and worked up according to the standard procedure: yield of 1-(4-fluorophenyl)-1*H*-indole-3-carboxaldehyde 7.5 g (51%); mp 126–128 °C (diisopropyl ether); ^{1}H NMR ($CDCl_3$) δ 7.25 (t, 2H), 7.30–7.45 (m, 3H), 7.60 (dd, 2H), 7.85 (s, 1H), 8.35–8.45 (m, 1H), 10.10 (s, 1H). To a mixture of the indolecarboxaldehyde (3.0 g, 0.013 mol), spiro[isobenzofuran-1(3*H*),4'-piperidine] (1.6 g, 0.0084 mol), and sodium cyanoborohydride (4.0 g, 0.064 mol) in dry methanol (16 mL) was added molecular sieve powder (3 Å) (5 g). After the mixture was stirred for 16 h at room temperature, water (200 mL) and ethyl acetate (100 mL) were added. The organic phase was separated and worked up according to the standard procedure. The crude title product was purified by column chromatography on silica gel (eluted with 4% triethylamine in ethyl acetate and heptane, 1:1). The fumarate salt crystallized from ethanol: yield of 14i 1.3 g (28%); mp 243–244 °C; ^{1}H NMR (DMSO-d_6) δ 1.70 (broad d, 2H), 2.10 (dt, 2H), 2.70 (t, 2H), 3.10 (broad d, 2H), 4.05 (s, 2H), 4.95 (s, 2H), 6.60 (s, 2.5H), 7.15–7.25 (m, 6H), 7.40 (t, 2H), 7.45 (d, 1H), 7.60–7.70 (m, 3H), 7.90 (d, 1H). Anal. ($C_{27}H_{25}FN_2O$·1.25 fumarate) C, H, N.

1'-[4-[1-(3-Thienyl)-1*H*-indol-3-yl]-1-butyl]spiro[isobenzofuran-1(3*H*),4'-piperidine] Fumarate (14j). A mixture of 1'-[4-(1*H*-indol-3-yl)-1-butyl]spiro[isobenzofuran-1(3*H*),4'-piperidine], 12a (3.0 g, 0.0083 mol), 3-bromothiophene (3.8 g, 0.023 mol), potassium carbonate (1.6 g, 0.012 mol), CuI (0.5 g), and ZnO (0.2 g) in NMP (40 mL) was heated at 160 °C for 7 h. Inorganic salts were filtered off, and ethyl acetate (200 mL) and diluted aqueous NH_4OH (500 mL) were added. The organic phase was separated and worked up according to the standard procedure. Column chromatography on silica gel eluted with 4% triethylamine in ethyl acetate and heptane, 1:1) provided the pure title compound. The fumarate salt crystallized from ethanol: yield of 14j 1.1 g (24%); mp 183 °C; ^{1}H NMR (DMSO-d_6) δ 1.60–1.80 (m, 6H), 2.05 (dt, 2H), 2.55–2.85 (m, 6H), 3.05 (broad d, 2H), 5.00 (s, 2H), 6.60 (s, 2H), 7.10–7.30 (m, 7H), 7.50 (d, 1H), 7.50 (s, 1H), 7.55–7.65 (m, 1H), 7.75–7.80 (m, 1H). Anal. ($C_{28}H_{30}N_2OS$·fumarate) C, H, N.

In a corresponding way the following 1-heteroaryl-substituted indoles 14 were prepared.

1'-[4-[1-(2-Thienyl)-1*H*-indol-3-yl]-1-butyl]spiro[isobenzofuran-1(3*H*),4'-piperidine] oxalate (14k): mp 198–201 °C (acetone); ^{1}H NMR (DMSO-d_6) δ 1.70–1.90 (m, 6H), 2.20 (dt, 2H), 2.80 (t, 2H), 3.00–3.20 (m, 4H), 3.50 (broad d, 2H), 5.05 (s, 2H), 7.10–7.35 (m, 8H), 7.45 (d, 1H), 7.45 (s, 1H), 7.60 (d, 1H), 7.65 (d, 1H). Anal. ($C_{28}H_{30}N_2OS$·oxalate) C, H, N.

1'-[4-[1-(2-Thiazolyl)-1*H*-indol-3-yl]-1-butyl]spiro[isobenzofuran-1(3*H*),4'-piperidine] fumarate (14l): mp 165–167 °C (ethanol); ^{1}H NMR (DMSO-d_6) δ 1.55–1.85 (m, 6H), 1.95 (dt, 2H), 2.55–2.65 (m, 4H), 2.75 (t, 2H), 2.95 (broad d, 2H), 4.95 (s, 2H), 6.55 (s, 2H), 7.15–7.30 (m, 5H), 7.40 (t, 1H), 7.45 (d, 1H), 7.60–7.70 (m, 3H), 8.25 (d, 1H). Anal. ($C_{27}H_{29}N_3S$·fumarate) C, H, N.

1'-[4-[1-(4-Pyridyl)-1*H*-indol-3-yl]-1-butyl]spiro[isobenzofuran-1(3*H*),4'-piperidine] dioxalate (14m): mp 127–129 °C (acetone); ^{1}H NMR (DMSO-d_6) δ 1.70–1.90 (m, 6H), 2.25 (broad t, 2H), 2.80 (t, 2H), 3.10–3.30 (m, 4H), 3.50 (broad d, 2H), 5.05 (s, 2H), 7.15–7.35 (m, 6H), 7.65–7.75 (m, 3H), 7.85 (d, 1H), 8.65 (d, 2H). Anal. ($C_{29}H_{31}N_3O$·dioxalate) C, H, N.

1-(4-Fluorophenyl)-3-[4-[3-(4-fluorophenyl)-8-azabicyclo[3.2.1]oct-2-en-8-yl]-1-butyl]-1*H*-indole (15a). A mixture of the methanesulfonate ester (6.0 g, 0.016 mol) of 4-[1-(4-fluorophenyl)-1*H*-indol-3-yl]-1-butanol, 10a, prepared as described in the synthesis of compound 11a, 3-(4-fluorophenyl)-8-azabicyclo[3.2.1]oct-2-ene (4.0 g, 0.020 mol), and potassium carbonate (5.0 g, 0.036 mol) was heated at reflux temperature in MIBK (80 mL) for 16 h. After cooling, inorganic salts were filtered off, and MIBK was evaporated in vacuo. The remaining product was purified by column chromatography on silica gel (eluted with 4% triethylamine in ethyl acetate and heptane, 2:3). The pure title compound crystallized from diisopropyl ether: yield of 15a 1.9 g (25%); mp 74 °C; ^{1}H NMR ($CDCl_3$) δ 0.85–2.20 (m, 9H), 2.55 (t, 2H), 2.75–2.80 (m, 1H), 2.80 (t, 2H), 3.45 (q, 2H), 6.20 (d, 1H), 6.90 (t, 2H), 7.00 (s, 1H), 7.10–7.20 (m, 4H), 7.25–7.35 (m, 2H), 7.35–7.45 (m, 3H), 7.60 (d, 1H). Anal. ($C_{31}H_{30}F_2N_2$) C, H, N.

Pharmacological Test Methods. Animals for Binding. Male Wistar rats (Mol:Wist, SPF, 170–270 g) were used. We have recently described the handling proceduces in detail.[38]

Animals for Behavior. Male Wistar WU rats (Charles River, Germany, 200–250 g) were used.

Calculations. ED_{50} values were calculated by log–probit analyses. IC_{50} values were estimated from concentration–effect curves using a log–concentration scale. Details are available from the references cited in the description of specific test methods below.

Black/White Box Exploration Test. This test model is a modified version of the model reported by Colpaert et al.[39] Our modifictions have recently been described in detail.[40] The σ ligands were administered subcutaneously 2 h before the test session or as indicated in this paper.

Binding σ Binding Sites In Vitro. σ_1 Site. Affinity of test compounds to σ_1 binding sites was estimated by their ability to displace [^{3}H]-(+)-pentazocine from rat brain homogenates minus cerebellum, as described by DeHaven-Hudkins et al.[41]

σ_2 Site. Affinity of test compounds to σ_2 binding sites was estimated by their ability to displace [^{3}H]-1,3-di(2-tolyl)-guanidine (DTG) from rat brain homogenates minus cerebellum, as described by Sonesson et al.[30]

Receptor Binding in Vitro. DA D_2 Receptors. Affinity of test compounds to dopamine D_2 receptors was estimated by their ability to displace [^{3}H]spiperone from rat striatal membranes, as described by Hyttel.[42]

5-HT_{2A} Receptors. Affinity of test compounds to serotonin 5-HT_{2A} receptors was estimated by their ability to displace [^{3}H]-ketanserin from rat cortical membranes, as described by Hyttel.[42]

5-HT_{1A} Receptors. Affinity of test compounds to serotonin 5-HT_{1A} receptors was estimated by their ability to displace [^{3}H]-8-OH-DPAT from whole rat brain membranes minus cerebellum, as described by Hyttel et al.[43]

α_1 Adrenoceptors. Affinity of test compounds to α_1 adrenoceptors was estimated by their ability to displace [^{3}H]-prazosin from whole rat brain membranes, as described by Skarsfeldt and Hyttel.[44]

Acknowledgment. We acknowledge the very skillful technical assistance of J. W. Stenberg and B. Hansen for the syntheses of compounds. The preparation of the manuscript by Mrs. S. Henriksen is highly appreciated. Finally, we thank all from the staff of the Lundbeck Research Departments who have contributed to the fulfillment of the present study.

References

(1) Su, T. P. Evidence for σ Opoid Receptor Binding of [^{3}H]SKF10047 to Etorphine-Inaccessible Sites in Guinea Pig Brain. *J. Pharmacol. Exp. Ther.* **1982**, *223*, 284–290.
(2) Tam, S. W. Naloxone-Inaccessible Sigma Receptor in Rat Central Nervous System. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 6703–6707.
(3) Ferris, C. D.; Hirsch, D. J.; Brooks, B. P.; Snyder, S. H. σ Receptors: From Molecule to Man. *J. Neurochem.* **1991**, *57*, 729–737.
(4) Quirion, R.; Bowen, W. D.; Itzhak Y.; Junien, J. L.; Musacchio J. M.; Rothman R. B.; Su, T. B.; Tam, W.; Taylor, D. P. A Proposal for the Classification of Sigma Binding Sites. *Trends Pharmacol. Sci.* **1992**, *13*, 85–86.

(5) Bowen, W. D.; Hellewell, S. B.; McGarry, K. A. Evidence for a Multisite Model of the Rat Brain σ Receptor. *Eur. J. Pharmacol.* 1989, *163*, 309–318.
(6) Mewshaw, R. E.; Sherill, R. G.; Mathew, R. M.; Kaiser, C.; Bailey, M. A.; Karbon, E. W. Synthesis and in Vitro Evaluation of 5,6,7,8,9,10-Hexahydro-7,10-imino-cyclohept[b]indoles: High Affinity Ligands for the N,N'-di-o-tolylguanidine-labeled σ Binding Site. *J. Med. Chem.* 1993, *36*, 343–352.
(7) deCosta, B. R.; He, X.; Dominguez, C.; Cutts, J.; Williams, W.; Bowen, W. D. A New Approach to the Design of σ_2 Selective Ligands: Synthesis and Evaluation of N-[2-(3,4-Dichlorophenyl)-ethyl]-N-methyl-2-(1-pyrrolidinyl)ethylamine-Related Polyamines at σ-1 and σ-2 Receptor Subtypes. *J. Med. Chem.* 1994, *37*, 314–321.
(8) Bertha, C. M.; Vilner, B. J.; Williams, W.; Rice, K. C., Bowen, W. D. *E*-8-Benzylidene-2-methyl-5-phenylmorphans: A Novel Class of High Affinity Ligands Which Exhibit Sigma-1 or Sigma-2 Subtype Selectivity. Soc.Neurosci.. 1994, *20*, abstract no. 314.2.
(9) Ferris, R. M.; Tang, F. L. M.; Chang, K.-J.; Russell, A. Evidence that the Potential Antipsychotic Agent Rimcazole (BW 234U) is a Specific, Competitive Antagonist of Sigma Sites in Brain. *Life Sci.* 1986, *38*, 2329–2337.
(10) Davidson, J.; Miller, R.; Wingfield, M.; Zung, M.; Dren, A. T. The First Clinical Study of BW-234U in Schizophrenia. *Psychopharmacol. Bull.* 1982, *18*, 173–176.
(11) Chouinard, F.; Annable, L. An Early Phase II Clinical Trial of BW234U in the Treatment of Acute Schizophrenia in Newly Admitted Patients. *Psychopharmacology* 1984, *84*, 282–284.
(12) Bellville, J. W.; Forrest, H., Jr. Respiratory and Subjective Effects of d- and l-Pentazocine. *Clin. Pharmacol. Ther.* 1968, *9*, 142–151.
(13) Lai, N. L.; Bowen, W. D.; Matsumoto, R. R.; Thurkauf, A.; Rice, K. C.; Walker, J. M. Anxiogenic Effects of two Selective Sigma Ligands in the Rat. *Soc. Neurosci.* 1989, *15*, abstract no. 270.9.
(14) Taylor, D. P.; Dekleva, J. Potential Antipsychotic BMY 14802 Selectively Binds to Sigma Sites. *Drug Dev. Res.* 1987, *11*, 65–70.
(15) Gilligan, P. J.; Cain, G. A.; Christos T. E.; Cook, L.; Drummond, S.; Johnson, A. L.; Kergaye, A. A.; McElroy, J. F.; Rohrbach, K. W.; Schmidt, W. K.; Tam, S. W. Novel Piperidine σ Receptor Ligands as Potential Antipsychotic Drugs. *J. Med. Chem.* 1992, *35*, 4344–4361.
(16) Cook, L.; Tam, S. W.; Rohrbach, K. W. DuP 734 [1-(Cyclopropylmethyl)-4-(2'-(4"-fluorophenyl)-2'-oxoethyl)piperidine Hydrobromide], a Potential Antipsychotic: Preclinical Behavioral Effects. *J. Pharmacol. Exp. Ther.* 1992, *263*, 1159–1166.
(17) Perregaard, J.; Stenberg, J. W. Preparation of Piperazinyl Derivatives, and Their Use as Serotonergic Agonists in the Treatment of Central Nervous System Disorders. U.S. Pat. No. 5,002,948, 1991; *Chem. Abstr.* 1990, *114*, 17582m.
(18) Perregaard, J.; Arnt, J.; Hyttel, J. 3-Indolylbutyl- and 3-(2,3-Dihydroindolyl)butylpiperazines as Partial 5-HT_{1A} Agonists. Poster presented at 8th Camerino-Noordwijkerhout Symposium, 8–12 Sept 1991, Camerino, Italy; Abstract no. P34.
(19) Ablordeppey, S. Y.; Issa, H.; Fischer, J. B.; Howie, K. J. B.; Glennon, R. A. Synthesis and Structure-Affinity Relationship Studies of Sigma Ligands Related to Haloperidol. *Med. Chem. Res.* 1993, *3*, 131–138.
(20) Schuster, D. I.; Pan, Y.; Singh, G.; Stoupakis, G.; Cai, B.; Lem, G.; Ehrlich, G. K.; Frietze, W.; Murphy, R. B. N-(1-Arylpropionyl)-4-aryltetrahydropyridines, a New Class of High-Affinity Selective σ Receptor Ligands. *J. Med. Chem.* 1993, *36*, 3923–3928.
(21) Jaen, J. C.; Caprathe, B. W.; Pugsley, T. A.; Wise, L. D.; Akunne, H. Evaluation of the Effects of the Enantiomers of Reduced Haloperidol, Azaperol, and Related 4-Amino-1-arylbutanols on Dopamine and σ receptors. *J. Med. Chem.* 1993, *36*, 3929–3936.
(22) Bowen, W. D.; Moses, E. L.; Tolentino, P. J.; Walker, J. M. Metabolites of Haloperidol Display Preferential Activity at σ Receptors Compared to Dopamine D-2 Receptors. *Eur. J. Pharmacol.* 1990, *177*, 111–118.
(23) Böttcher, H.; Barnickel, G.; Hausberg, H.-H.; Hasse, A. F.; Seyfried, C. A.; Eiermann, V. Synthesis and Dopaminergic Activity of Some 3-(1,2,3,6-Tetrahydro-1-pyridylalkyl)indoles. A Novel Conformational Model to Explain Structure-Activity Relationships. *J. Med. Chem.* 1992, *35*, 4020–4026.
(24) Benghiat, E., Crooks, P. A. Multisubstrate Adducts as Potential Inhibitors of *S*-Adenosylmethionine Dependent Methylases: Inhibition of Indole N-Methyltransferase by (5'-Deoxyadenosyl)-[3-(3-indolyl)prop-1-yl]methylsulfonium and N-Methyltransferase by (5'-Deoxyadenosyl)[4-(3-indolyl)but-1-yl]methylsulfonium Salts. *J. Med. Chem.* 1983, *26*, 1470–1477.
(25) Manallack, D. T.; Wong, M. G.; Costa, M.; Andrews, P. R.; Beart, P. M. Receptor Site Topographics for Phencyclidine-Like and σ Drugs: Predictions from Quantitative Conformational, Electrostatic Potential, and Radioreceptor Analyses. *Mol. Pharmacol.* 1988, *34*, 863–879.
(26) Hibert, M. F.; Gittos, M. W.; Middlemiss, D. N.; Mir, A. K.; Fozard, J. R. Graphics Compouter-Aided Receptor Mapping as a Predictive Tool for Drug Design: Development of Potent, Selective, and Stereospecific Ligands for the 5-HT_{1A} Receptor *J. Med. Chem.* 1988, *31*, 1087–1093.
(27) Hibert, M. F.; McDermott, I.; Middlemiss, D. N.; Mir, A. K.; Fozard, J. R. Radio-ligand Binding Study of a Series of 5-HT_{1A} Receptor Agonists and Definition of a Steric Model of This Site. *Eur. J. Med. Chem.* 1989, *24*, 31–37.
(28) Marxer, A.; Rodriguez, H. R.; McKenna, J. M.; Tsai, H. M. Spiropiperidines. I. Synthesis of Spiro[isobenzofuran-1(3H),4'-piperidines] and Spiro[isobenzofuran-1(3H),3'-piperidines]. *J. Org. Chem.* 1975, *40*, 1427–1433.
(29) Elderfield, R. C.; Fischer, B.; Lagowski, J. M. Action of Metal Hydrides on β-(3-Indolyl)ethyl-1-pyridinium Salts. *J. Org.Chem.* 1957, *22*, 1376–1380.
(30) Sonesson, C.; Waters, N.; Svensson, K.; Carlsson, A.; Smith, M. W.; Piercey, M. F.; Meier, E.; Wikström, H. Substituted 3-Phenylpiperidines: New Cnetrally Acting Dopamine Autoreceptor Antagonists. *J. Med. Chem.* 1993, *36*, 3188–3196.
(31) Chambers, M. S.; Baker, R.; Billington, D. C.; Knight, A. K.; Middlemiss, D. N.; Wong, E. H. F. Spiropiperidines as High-Affinity, Selective σ Ligands. *J. Med. Chem.* 1992, *35*, 2033–2039.
(32) Bristow, L. J.; Baucutt, L.; Thorn, L.; Huton, P. H.; Noble, A.; Beer, M.; Middlemiss, D. N.; Tricklebank, M. D. Behavioral and Biochemical Evidence of the Interaction of the Putative Antipsychotic Agent BMY 14802 with the 5-HT_{1A} Receptor. *Eur. J. Pharmacol.* 1991, *204*, 21–28.
(33) Burns, H. D.; Brenner, N. J.; Dannals, R. F.; Gibson, R. E.; Wilson, A. A.; Ravert, H. T.; Chambers, M.; Middlemiss, D. N.; Wong, D. F.; Wagner, H. N., Jr. Synthesis of a Radiotracer for Studying Sigma Recognition Sites Using Positron Emission Tomography Carbon-14 L-687384. *J. Labelled Compd. Radiopharm.* 1993, *32*, 338–339.
(34) Moltzen, E. K.; Perregaard, J.; Meier, E.; Sánchez, C.; Arnt, J.; Nielsen, J. B. Spirocyclic Isobenzofuran Derivatives: a New Class of High-Affinity Sigma Ligands with Potent Anxiolytic Activities. Poster no.P-105-A Presented at XIIth International Symp. on Medicinal Chemistry, Basel, Sept 13–17, 1992.
(35) Perregaard, J.; Moltzen, E. K.; Meier, E.; Sánchez, C.; Hyttel, J. 4-Phenylpiperidines and 4-Spiropiperidines with Subnanomolar Affinity for Sigma Binding Sites and with Potent Anxiolytic Activity. *Soc. Neurosci.* 1993, *19*, abstract no. 763.16.
(36) Sánchez, C.; Arnt, J.; Perregaard, J. Lu 28-179: A Selective Sigma Ligand with Potent Anxiolytic Effects. *Soc. Neurosci.* 1994, *20*, abstract no. 164.16. Manuscript in preparation.
(37) McElvain, M. S.; Safranski, J. C. Piperidine derivatives. XXIII. Certain haloge-nated 1-methyl-4-phenylpiperidines and Related Compounds. *J. Am. Chem. Soc.* 1950, *72*, 3134–3138.
(38) Sánchez, C.; Arnt, J.; Dragsted, N.; Hyttel, J.; Lembøl, H. L.; Meier, E.; Perregaard, J.; Skarsfeldt, T. Neurochemical and In Vivo Pharmacological Profile of Sertindole, a Limbic-Selective Neuroleptic Compound. *Drug Dev. Res.* 1991, *22*, 239–250.
(39) Colpaert, F. C.; Meert, T. F.; Niemegeers, C. J. E.; Janssen, P. A. J. Behavioral and 5-Hydroxytryptamine Antagonist Effects of Ritanserin. A Pure and Selective Antagonist of LSD Discrimination in Rat. *Psychopharmacology* 1985, *86*, 45–54.
(40) Sánchez, C.; Arnt, J.; Costall, B.; Domeney, A. M.; Kelly, E.; Naylor, R. J. Sertindole: A Limbic Selective Neuroleptic with Potent Anxiolytic Effects. *Drug Dev. Res.* 1995, *34*, 19–29.
(41) DeHaven-Hudkins, D. L.; Fleissner, L. C.; Ford-Rice, F. Y. Characterization of the Binding of [^{3}H](+)-Pentazocine to σ Recognition Sites in Guinea Pig Brain. *Eur. J. Pharmacol.—Mol. Pharmacol. Soc.* 1992, *227*, 371–378.
(42) Hyttel, J. Age Related Decrease in the Density of Dopamine D_1 and D_2 Receptors in Corpus Striatum of Rats. *J. Pharmacol. Toxicol.* 1987, *61*, 126–129.
(43) Hyttel, J.; Bøgesø, K.; Lembøl, H. L.; Larsen, J.-J.; Meier, E. Neurochemical Profile in Vitro of Irindalone: A 5-HT2-Receptor Antagonist. *Drug Dev. Res.* 1988, *15*, 389–404.
(44) Skarsfeldt, T.; Hyttel, J. The St 587-Induced Flexor Reflex in Pithed Rats: A Model to Evaluate Central α_1-receptor Blocking Properties. *Eur. J. Pharmacol.* 1986, *125*, 333–340.

JM940856C